IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Appellants:	Michael T. Lotze *et al.*
Title:	*METHODS AND REAGENTS FOR INDUCING IMMUNITY*
Appl. No.:	10/688,845
Filing Date:	10/15/03
Examiner:	Amy E. Juedes
Art Unit:	1644
Confirmation Number:	9535

BRIEF ON APPEAL

Mail Stop Appeal Brief - Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Under the provisions of 37 C.F.R. § 41.37, Appellants file this Appeal Brief. A Notice of Appeal was filed July 10, 2008, with a credit card payment form in the amount of $510, covering the fee mandated by 37 C.F.R. 41.20(b)(2). Appellants encloses a Petition for Extension of Time to make this Appeal Brief timely.

If any additional fees necessary to timely file this Appeal Brief are deemed insufficient, authorization is hereby given to charge any deficiency (or credit any balance) to the undersigned deposit account 19-0741.

11/12/2008 JADD01 00000019 10688845
01 FC:1402 540.00 OP
02 FC:1252 490.00 OP

TABLE OF CONTENTS

REAL PARTY IN INTEREST

The real parties in interest are the University of Pittsburgh, the assignee of this application, and Intrexon Corp., a licensee.

STATUS OF CLAIMS

Claims 1-31 and 35-40 are pending, and claims 1-26, 30 and 39 are withdrawn. Claims 32-34 were cancelled without prejudice or disclaimer.

Claims 27-29, 31, 35-38, and 40 stand rejected and are being appealed.

SUMMARY OF CLAIMED SUBJECT MATTER

Two independent claims, claims 27 and 36, are on appeal. Appellants provide below "a concise explanation of the subject matter defined in each of the independent claims involved in the appeal, referring to the specification by page and line number." *See* 37 C.F.R. § 41.37.

Claim 27 is directed to a therapeutic composition comprising an antigen presenting cell and an immunostimulalatory cytokine or nucleic acid encoding an immunostimulatory cytokine. *See* specification at page 3, paragraph [0008] The antigen presenting cell is not loaded or pulsed with antigens. *Id.* at ¶ [0027]. The therapeutic composition also comprises a pharmaceutically acceptable solution or buffer. *Id.* at ¶ [0040]. The specification explains that these therapeutic compositions can be used to treat tumor growth, metastasis, or infection. *Id.* at page 4, paragraph [0008].

Claim 36 is directed to a therapeutic composition comprising a pharmaceutically acceptable carrier, an antigen presenting cell, and an immunostimulatory cytokine or nucleic acid encoding an immunostimulatory cytokine. *Id.* at page 4, paragraph [0009]. The antigen presenting cell is not loaded or pulsed with antigens. *Id.* at ¶ [0027]. The specification explains that these formulations can be used to treat a subject having a tumor, metastasis, or infection lesion. *Id.* at page 16, paragraph [0042].

GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

This appeal presents two grounds of rejection for review:

(1) the rejection of claims 27-29, 31, 35-38, and 40 under 35 U.S.C. § 102(b), as allegedly anticipated by Bhardwaj *et al.*, J. CLIN. INVEST. 98:715-722 (1996), as evidenced by Hackstein *et al.*, BLOOD 100(3):1084-1087 (2002); and

(2) the rejection of claims 27-29, 31, 35-38, and 40 under 35 U.S.C. § 102(b), as allegedly anticipated by Kelleher *et al.*, INT'L IMMUNOLOGY 10(6):749-755 (1998).

ARGUMENT

The rejections should be withdrawn as both legally and factually flawed. The claims are directed to a "therapeutic composition." In rejecting the claims, the Examiner disregards the preamble recitation of a "therapeutic composition." Yet, in this instance the preamble breathes life and meaning into the claims, and Appellants relied on and continue to rely on the preamble to distinguish the prior art. Accordingly, the Examiner's disregard of the preamble is legal error.

The Examiner also concludes that the prior art compositions, which are simply cell cultures, are "therapeutic compositions" because they are "physiologically compatible." This conclusion is the manifestation of an unreasonable claim construction and is also contradicted by the evidence of record.

A. Preambles Can Limit Claim Scope

The determination of whether a preamble limits a claim is made on a case-by-case basis in light of the facts in each case. *Catalina Mktg. Int'l v. Coolsavings.com, Inc.*, 289 F.3d 801, 808, 62 USPQ2d 1781, 1785 (Fed. Cir. 2002). In performing an analysis, the entire claim should be considered along with the specification to determine what the inventors intended to be encompassed by the claim. *Corning Glass Works v. Sumitomo Electric U.S.A., Inc.*, 868 F.2d 1251, 1257 (Fed. Cir. 1989); *Pitney Bowes, Inc. v. Hewlett-Packard Co.*, 182 F.3d 1298, 1305, 51 USPQ2d 1161, 1165-66 (Fed. Cir. 1999).

The preamble should be limiting if it is "necessary to give life, meaning, and vitality" to the claim when read as a whole. *Pitney Bowes, Inc. v. Hewlett-Packard Co.*, 182 F.3d 1298, 1305 (Fed. Cir. 1999) (quotations omitted) (citations omitted). The repeated reference to the preamble in the specification and an indication that it constitutes an important characteristic of the invention is sufficient to make the preamble limiting. *Poly-America, L.P. v. GSE Lining Tech., Inc.*, 383 F.3d 1303, 1310 (Fed. Cir. 2004).

"[C]lear reliance on the preamble during prosecution to distinguish the claimed invention from the prior art transforms the preamble into a claim limitation because such reliance indicates use of the preamble to define, in part, the claimed invention." *Catalina Mktg. Int'l v. Coolsavings.com, Inc.*, 289 F.3d 801, 808 (Fed. Cir. 2002). The reliance can take the form of amending the claims to add the limitation. *Invitrogen Corp. v. Biocrest Mfg., L.P.*, 327 F.3d 1364, 1370 (Fed. Cir. 2003). The reliance can also be based on arguments distinguishing the prior art based on the preamble. *In re Cruciferous Sprout Litig.*, 301 F.3d 1343, 1348 (Fed. Cir. 2002).

B. "Therapeutic Composition" Serves As A Limitation (Claims 27-29, 31, 35-38 and 40)

When read on the proper context, one of skill in art would understand the term "therapeutic composition" to breath life and meaning into the claims. The specification is replete with references to the therapeutic importance of the claimed invention. Indeed, the Field of the Invention section states that "the invention is directed to the use of co-administration of antigen presenting cells and immunostimulatory cytokines ***to treat tumors or infections***." Spec. at ¶ [0003] (emphasis added). Consistent with the Field of the Invention section, the Summary of the Invention section states that the "instant invention relates to methods and reagents ***for treating a tumor or infection*** by administering an immunostimulatory cytokine, or a nucleic acid encoding an immunostimulatory cytokine, in combination with antigen presenting cells ***into or near a tumor or infectious lesion***." Spec. at ¶ [0007] (emphasis added). The remaining portions of the specification provide details on the therapeutic composition and how they can be administered to "treat[] a tumor or infection." Thus, the specification leaves no doubt. The invention is not directed to mere

cultures. Rather, the invention is directed to, *inter alia*, therapeutic compositions "to treat tumors or infections."

The claim language itself emphasizes that the "therapeutic composition" language breathes life and meaning into the claims. Claims 27 and 36 recite "a physiologically acceptable solution or buffer" and "a pharmaceutically acceptable carrier," respectively. Unless the claims were directed to "therapeutic compositions," there would be no reason for this language. Cell cultures do not need to include physiologically acceptable solutions or buffers or pharmaceutical carriers. This language only makes sense when considered with the "therapeutic composition" preamble. Thus, the claims themselves, when considered as a whole, confirm that the "therapeutic composition" language is intended to breath life and meaning into the claims. *Aventis Pharms., Inc. v. Barr Labs., Inc.*, 341 F. Supp. 2d 502, 509 (D.N.J. 2004) (holding "pharmaceutical composition" in preamble to be limiting).

Moreover, Appellants have repeatedly relied on the "therapeutic composition" language to distinguish the prior art, which discloses cell cultures with no suggestion of therapeutic applicability. Accordingly, Appellants have defined the claimed invention, in part, based on the preamble. Accordingly, the preamble constitutes a claim limitation. *Catalina Mktg. Int'l v. Coolsavings.com, Inc.*, 289 F.3d 801, 808 (Fed. Cir. 2002); *In re Cruciferous Sprout Litig.*, 301 F.3d 1343, 1348 (Fed. Cir. 2002).

C. The Prior Art Does Not Teach The Claimed "Therapeutic Composition"

1. The Prior Art Teaches Cell Cultures Rather Than Therapeutic Compositions

It is undisputed that the Bhardwaj and Kelleher do not teach or suggest a therapeutic application. Instead, Bhardwaj and Kelleher disclose cell cultures developed in the course of general scientific research. Specifically, Bhardwaj discloses a cell culture system that has both IL-12 and dendritic cells, which are either infected with influenza or uninfected. These compositions were used to evaluate the role of IL-12 in generating cytolytic T lymphocyte (CTL) responses to influenza virus. Kelleher discloses a cell culture system comprising dendritic cells. IL-12 was added to some of these cultures to determine "whether IL-12

administration during DC maturation altered cell numbers, phenotype and function." Kelleher at 749, right col. Neither of these references discloses a "therapeutic composition," as claimed.

One of skill in the art would understand a "therapeutic composition" as follows:

> a composition suitable for administration to a patient for the treatment of some disease or condition. Therapeutic compositions are formulated so as to preserve the stability of the active agents while making the composition biologically compatible. Thus, a therapeutic composition is more than just an active agent. A therapeutic composition contains one or more active agents formulated such that they can be safely administered to patients for the treatment of some disease or condition.

Declaration Under 37 C.F.R. § 1.132 of Michael T. Lotze ("Lotze Decl."), submitted June 29, 2007, at ¶ 7. Bhardwaj and Kelleher do not teach suggest a composition. Rather, the references teach cell cultures designed to explore the role of IL-12. Nothing in Bhardwaj and Kelleher teaches or suggests that the cultures have any therapeutic value, much less that they should be formulated to be safely administered to patients. Accordingly, Bhardwaj and Kelleher do not teach or suggest the claimed invention.

2. The Prior Art Cultures Are Incompatible With "Physiological Composition"

The Examiner contends that the cultures of Bhardwaj and Kelleher constitute "therapeutic compositions" because "[c]ells in culture are considered to be compatible with physiological conditions an not incompatible with therapeutical use." Office Action dated April 10, 2008 at ¶ 5. This contention fails for two reasons.

First, the contention is premised on a legally flawed claim construction. The broadest reasonable interpretation of claims must be used during examination, but "[t]he broadest reasonable interpretation of the claims must also be consistent with the interpretation that those skilled in the art would reach." MPEP § 2111. The broadest reasonable interpretation is based on the "ordinary and customary meaning" claim terms would have to one of ordinary to skill in the art. MPEP § 2111.01(II).

One of skill in the art understands that a "therapeutic composition" is a composition suitable for administration to a patient for the treatment of some disease or condition and contain an active agent along with addition components to preserve the active of the active agent. Lotze Decl. at ¶¶ 7, 9. Thus, even if cell culture may be "physiologically compatible," one of skill in the art would not necessarily understand that culture to be a "therapeutic composition." In order to be a "therapeutic composition," there must be some indication that the composition should actually be used in that manner (i.e. administered to a patient). *Id.* Accordingly, the broadest reasonable construction of "therapeutic composition" requires something more than mere physiological compatibility.

Second, even if a "therapeutic composition" could be reasonably construed to be any physiologically compatible composition, the prior art does not disclose a physiologically compatible composition.

Bhardwaj's cell culture is not "physiologically compatible" because Bhardwaj's crude culture contains agents, such as impurities, antithetical to a "therapeutic composition." Lotze Decl. at ¶ 10. Moreover, Bhadwaj's cell cultures are "infected with a live or heat-inactivated influenza virus." Bhardwaj, page 716, left column. Such a composition could cause serious complications if it were to be administered to a patient. Lotze Decl. at ¶ 10. Accordingly, one of skill in the art would consider Bhardwaj's cell culture to be unsuitable for use as a therapeutic agent. *Id.*

Nor is Kelleher's culture "physiologically compatible," because Kelleher's culture contains, "RPMI 1640 (Dutch modification; Sigma, Poole, UK) supplemented with 10% FCS (Gibco, Paisley, UK), 100 U Penicillin (Gibco), 100 μg/ml streptomycin (Gibco), 2 mM glutamine (ICN Flow, Irvine, UK), 2 mercaptoethanol 10^{-5} M (Sigma)." Kelleher, p. 750. One of skill in the art would understand that administration of several components Kelleher's culture, such as FCS (fetal calf serum) and 2-mercaptoethanol, could cause serious complications if it were to administration to a patient. Lotze Decl. at ¶ 10. Accordingly, the cultures of Bhardwaj and Helleher are not "physiologically compatible" as "therapeutic components."

D. Conclusion

Appellants respectfully request that the rejections of claims 27-29, 31, 35-38 and 40 be reversed because the rejections are unsupported by the law or the evidence of record.

Respectfully submitted,

Date November 10, 2007

By S. A. Bent

FOLEY & LARDNER LLP
Customer Number: 22428
Telephone: (202) 672-5404
Facsimile: (202) 672-5399

Stephen A. Bent
Attorney for Appellants
Registration No. 29,768

CLAIMS APPENDIX

1. (Withdrawn) A method of inhibiting or treating a tumor or infectious lesion in a subject, comprising: administering into or near a site of a tumor or infectious lesion in a subject an effective amount of an antigen presenting cell and an immunostimulatory cytokine or a nucleic acid encoding an immunostimulatory cytokine.

2. (Withdrawn) The method of claim 1, wherein the antigen presenting cell is a dendritic cell.

3. (Withdrawn) The method of claim 2, wherein the dendritic cell is selected from the group consisting of a CD34+-derived dendritic cell, a bone marrow-derived dendritic cell, a monocytederived dendritic cell, a splenocyte derived dendritic cell, a skin-derived dendritic cell, a follicular dendritic cell, and a germinal center dendritic cell.

4. (Withdrawn) The method of claim 1, wherein the dendritic cell is a CD34+-derived dendritic cell cultured in the presence of at least one factor selected from the group consisting of granulocyte colony stimulating factor, granulocyte macrophage colony stimulatory factor, tumor necrosis factor alpha, interleukin 4, the Flt-3 ligand, and the kit ligand.

5. (Withdrawn) The method of claim 1, wherein the antigen presenting cell is selected from a group consisting of a Langherhans' cell, an interdigitating cell, a B cell, and a macrophage.

6. (Withdrawn) The method of claim 1, wherein the immunostimulatory cytokine is selected from the group consisting of interleukin-lα., interleukin-lβ, interleukin-2, interleukin-3, interleukin-4, interleukin-6, interleukin-8, interleukin-9, interleukin-lO, interleukin-12, interleukin-18, interleukin-19, interleukin-20, interleukin-23, interleukin-27, interleukin-lf3, interleukin-1f5, interleukin-1f6, interleukin-lf7, interleukin-lf8, interleukin-lf9, interleukin-lfl0, interferon-α., interferon-β, interferon-γ, tumor necrosis factor α, transforming growth factor-β,

granulocyte colony stimulating factor, macrophage colony stimulating factor, granulocyte-macrophage colony stimulating factor, the Flt3 ligand, and the kit ligand.

7. (Withdrawn) The method of claim 1, wherein the expression vector is a viral vector.

8. (Withdrawn) The method of claim 2, wherein the expression vector is selected from the group consisting of an adenoviral vector, an adeno-associated viral vector, a retroviral vector, a lentiviral vector, a herpes viral vector, and a vaccinia viral vector.

9. (Withdrawn) The method of claim 1, wherein the subject has a tumor selected from the group consisting of melanoma, hepatoma, adenocarcinoma, colorectal cancer, basal cell cancer, oral cancer, nasopharyngeal cancer, laryngeal cancer, bladder cancer, head and neck cancer, renal cell cancer, pancreatic cancer, pulmonary cancer, cervical cancer, ovarian cancer, esophageal cancer, gastric cancer, prostate cancer, testicular cancer, and breast cancer.

10. (Withdrawn) The method of claim 1, wherein the size of the tumor or infectious lesion is decreased.

11. (Withdrawn) The method of claim 1, wherein said administering step comprises injecting into the tumor or infectious lesion.

12. (Withdrawn) The method of claim 1, wherein said administering step comprises injecting the subject within the same organ as the tumor or infectious lesion.

13. (Withdrawn) A method of inhibiting or treating metastasis of a tumor in a subject, comprising: administer into or near a site of a tumor in a subject an effective amount of an antigen presenting cell and an immunostimulatory cytokine or a nucleic acid encoding an immunostimulatory cytokine.

14. (Withdrawn) The method of claim 13, wherein the antigen presenting cell is a

dendritic cell.

15. (Withdrawn) The method of claim 14, wherein the dendritic cell is selected from the group consisting of a CD34+-derived dendritic cell, a bone marrow-derived dendritic cell, a monocyte-derived dendritic cell, a splenocyte derived dendritic cell, a skin-derived dendritic cell, a follicular dendritic cell, and a germinal center dendritic cell.

16. (Withdrawn) The method of claim 13, wherein the dendritic cell is a CD34+-derived dendritic cell cultured in the presence of at least one factor selected from the group consisting of granulocyte colony stimulating factor, granulocyte macrophage colony stimulatory factor, tumor necrosis factor alpha, interleukin 4, the Flt-3 ligand, and the kit ligand.

17. (Withdrawn) The method of claim 13, wherein the antigen presenting cell is selected from a group consisting of a Langherhans' cell, an interdigitating cell, a B cell, and a macrophage.

18. (Withdrawn) The method of claim 13, wherein the immunostimulatory cytokine is selected from the group consisting of interleukin-1α, interleukin-1β, interleukin-2, interleukin-3, interleukin-4, interleukin-6, interleukin-8, interleukin-9, interleukin-10, interleukin-12, interleukin-18, interleukin-19, interleukin-20, interleukin-23, interleukin-27, interleukin-1f3, interleukin-1f5, interleukin-1f6, interleukin-1f7, interleukin-1f8, interleukin-1f9, interleukin-1f10, interferon-α, interferon-β, interferon-γ, tumor necrosis factor α, transforming growth factor-β, granulocyte colony stimulating factor, macrophage colony stimulating factor, granulocyte-macrophage colony stimulating factor, the Flt3 ligand, and the kit ligand.

19. (Withdrawn) The method of claim 13, wherein the expression vector is a viral vector.

20. (Withdrawn) The method of claim 13, wherein the expression vector is selected from the group consisting of an adenoviral vector, an adeno-associated viral vector, a retroviral vector, a lentiviral vector, a herpes viral vector, and a vaccinia viral vector.

21. (Withdrawn) The method of claim 13, wherein the subject has a tumor selected from the group consisting of melanoma, hepatoma, adenocarcinoma, colorectal cancer, basal cell cancer, oral cancer, nasopharyngeal cancer, laryngeal cancer, bladder cancer, head and neck cancer, renal cell cancer, pancreatic cancer, pulmonary cancer, cervical cancer, ovarian cancer, esophageal cancer, gastric cancer, prostate cancer, testicular cancer, and breast cancer.

22. (Withdrawn) The method of claim 13, wherein the size of the tumor or infectious lesion is decreased.

23. (Withdrawn) The method of claim 13, wherein the size of the metastasis is decreased.

24. (Withdrawn) The method of claim 13, wherein the number of the metastases is

25. (Withdrawn) he method of claim 13, wherein said administering step comprises injecting into the tumor or infectious lesion.

26. (Withdrawn) The method of claim 13, wherein said administering step comprises injecting the subject within the same organ as the tumor or infectious lesion.

27. (Previously Presented) A therapeutic composition comprising (a) a physiologically acceptable solution or buffer, (b) an antigen presenting cell, and (c) an immunostimulatory cytokine or a nucleic acid encoding an immunostimulatory cytokine, wherein the antigen presenting cell is not loaded or pulsed with antigens.

28. (Previously Presented) The composition of claim 27, wherein the antigen presenting cell is a dendritic cell.

29. (Previously Presented) The composition of claim 28, wherein the dendritic cell is selected from the group consisting of a CD34+-derived dendritic cell, a bone marrow-derived dendritic cell, a monocyte-derived dendritic cell, a splenocyte derived dendritic cell, a skin-derived dendritic cell, a follicular dendritic cell, and a germinal center dendritic cell.

30. (Withdrawn) The composition of claim 27, wherein the antigen presenting cell is selected from a group consisting of a Langherhans' cell, an interdigitating cell, a B cell, and a macrophage.

31. (Previously Presented) The composition of claim 27, wherein the immunostimulatory cytokine is selected from the group consisting of interleukin-1α, interleukin-1β, interleukin-2, interleukin-3, interleukin-4, interleukin-6, interleukin-8, interleukin-9, interleukin-10, interleukin-12, interleukin-18, interleukin-19, interleukin-20, interleukin-23, interleukin-27, interleukin-1f3, interleukin-1f5, interleukin-1f6, interleukin-1f7, interleukin-1f8, interleukin-1f9, interleukin-1f10, interferon-α, interferon-β, interferon-γ, tumor necrosis factor α, transforming growth factor-β, granulocyte colony stimulating factor, macrophage colony stimulating factor, granulocyte-macrophage colony stimulating factor, the Flt-3 ligand, and the kit ligand.

32.-34. (Canceled).

35. (Previously Presented) The composition of claim 27, further comprising a carrier.

36. (Previously Presented) A therapeutic composition comprising (a) a pharmaceutically acceptable carrier, (b) an antigen presenting cell, and (c) an immunostimulatory cytokine or a nucleic acid encoding an immunostimulatory cytokine, wherein the antigen presenting cell is not loaded or pulsed with antigens.

37. (Previously Presented) The composition of claim 36, wherein the antigen presenting cell is a dendritic cell.

38. (Previously Presented) The composition of claim 37, wherein the dendritic cell is selected from the group consisting of a CD34+-derived dendritic cell, a bone marrow-derived dendritic cell, a monocyte-derived dendritic cell, a splenocyte derived dendritic cell, a skin-derived dendritic cell, a follicular dendritic cell, and a germinal center dendritic cell.

39. (Previously Presented) The composition of claim 36, wherein the antigen presenting cell is selected from a group consisting of a Langherhans' cell, an interdigitating cell, a B cell, and a macrophage.

40. (Previously Presented) The composition of claim 36, wherein the immunostimulatory cytokine is selected from the group consisting of interleukin-1α, interleukin-1β, interleukin-2, interleukin-3, interleukin-4, interleukin-6, interleukin-8, interleukin-9, interleukin-10, interleukin-12, interleukin-18; interleukin-19, interleukin-20, interleukin-23, interleukin-27, interleukin-1f3, interleukin-1f5, interleukin-1f6, interleukin-1f7, interleukin-1f8, interleukin-1f9, interleukin-1f10, interferon-α, interferon-β, interferon-γ, tumor necrosis factor α, transforming growth factor-β, granulocyte colony stimulating factor, macrophage colony stimulating factor, granulocyte-macrophage colony stimulating factor, the Flt3 ligand, and the kit ligand.

EVIDENCE APPENDIX

Declaration Under 37 C.F.R. § 1.132 of Michael T. Lotze submitted June 29, 2007.

Bhardwaj *et al.*, J. CLIN. INVEST. 98:715-722 (1996).

Hackstein *et al.*, BLOOD 100(3):1084-1087 (2002).

Kelleher *et al.* INT'L IMMUNOLOGY 10(6):749-755 (1998).

Atty. Dkt. No. 076333-0393
Appl. No. 10/688,845

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Michael T. LOTZE et al.
Title:	METHODS AND REAGENTS FOR INDUCING IMMUNITY
Appl. No.:	10/688,845
Filing Date:	10/15/2003
Examiner:	Amy E. Juedes
Art Unit:	1644
Confirmation Number:	9535

DECLARATION UNDER 37 C.F.R. § 1.132 OF MICHAEL T. LOTZE

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Michael T. Lotze, declare as follows:

1. I am one of the inventors named on the captioned application.

2. My education includes a B.Med. Sciences, as well as an M.D., from Northwestern University and its Medical School.

3. I am a board licensed physician with board certifications in surgery and surgical oncology. Currently, I am Professor of Surgery and Bioengineering; Associate Director UPCI for Strategic Partnerships; Vice Chair of Research in the Department of Surgery; and Assistant Vice Chancellor for Sponsored Training Grants, University of Pittsburgh Schools of the Health Sciences.

4. In addition to my clinical duties, I perform research regarding the role of the immune system in cancer and the potential for using immune responses to treat cancer. I am

currently most interested in using natural killer (NK) cells and dendritic cells for cancer therapy. We have a National Cancer Institute funded Program Project Grant in this area.

5. My curriculum vitae is attached as Exhibit A.

6. I have read and understand the Office Action dated March 7, 2007 regarding the captioned application. I understand that the Office Action contends that claims 27-29 and 31-35 are anticipated by Bhardwaj *et al.*, J. CLIN. INVEST. 98:715-722 (1996) as evidenced by Hackstein *et al.*, BLOOD 100(3):1084-1087 (2002) and Kelleher *et al.* INT'L IMMUNOLOGY 10(6):749-755 (1998). I disagree.

7. Physicians and medical researchers understand a "therapeutic composition" to be a composition suitable for administration to a patient for the treatment of some disease or condition. Therapeutic compositions are formulated so as to preserve the stability of the active agents while making the composition biologically compatible. Thus, a therapeutic composition is more than just an active agent. A therapeutic composition contains one or more active agents formulated such that they can be safely administered to patients for the treatment of some disease or condition.

8. Turning to the references cited by the Office Action, Bhardwaj and Kelleher disclose cell cultures generated as part of research. More specifically, Bhardwaj discloses a cell culture that has both IL-12 and dendritic cells, which are either infected with influenza or uninfected. These compositions were used to evaluate the role of IL-12 in generating cytolytic T lymphocyte (CTL) responses to influenza virus. Kelleher discloses cultures comprising dendritic cells. IL-12 was added to some of these cultures to determine "whether IL-12 administration during DC maturation altered cell numbers, phenotype and function." Kelleher at 749, right col. The dendritic cells were cultured in "RPMI 1640 (Dutch modification; Sigma, Poole, UK) supplemented with 10% FCS (Gibco, Paisley, UK), 100 U Penicillin (Gibco), 100 μg/ml streptomycin (Gibco), 2 mM glutamine (ICN Flow, Irvine, UK), 2 mercaptoethanol 10^{-5} M (Sigma)." *Id.*

9. Physicians and medical researchers would not understand Bhardwaj and Kelleher to disclose a "therapeutic composition." Bhardwaj and Kelleher disclose cell

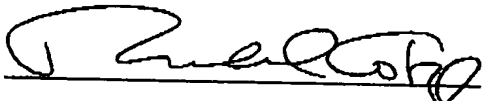
cultures, as I discussed above. Nothing in these references suggests that the cell cultures have any therapeutic value. The cultures were simply used to investigate the role of IL-12. Accordingly, a physician reading Bhardwaj and Kelleher would not consider their cell cultures to be "therapeutic compositions."

10. In addition, one of skill in the art would not consider the cultures of Bhardwaj and Kelleher to be "compatible with physiological conditions." These raw cell cultures would contain contaminants and impurities that may cause potentially serious reactions in patients. Many components of raw cell cultures have inhibitory proteins that are known, such as IL10 or TGFβ, or other elements which are unknown. Accordingly, a physician would not consider the cell cultures of Bhardwaj and Kelleher to be suitable for use "therapeutic composition[s]."

* * * * *

I hereby declare that all the statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful false statements are so made punishable by fine or imprisonment, or both, under Section 101 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: June 29, 2007

By: 

Michael T. Lotze, M.D.
Professor of Surgery and Bioengineering
Asst. Vice Chancellor, UPSHS
Vice Chair of Research, Department of Surgery
Director Strategic Partnerships, Univ. of Pittsburgh Cancer Institute
G.27A Hillman Cancer Center
5117 Centre Ave
Pittsburgh, PA 15213

CURRICULUM VITAE

Name: Michael T. Lotze, M.D.
Home Address: 5134 Westminster Place
Pittsburgh, PA 15232

Home Phone: 412-682-1514

Business: Professor Surgery, Molecular Genetics and Biochemistry; Bioengineering
University of Pittsburgh School of Medicine

Address: University of Pittsburgh Molecular Medicine Institute
Rm 411, 300 Technology Dr.
Pittsburgh, PA 15219

EDUCATION AND TRAINING

Undergraduate		
1969-1973	Northwestern University Evanston, IL	B. Medical Sciences
Graduate		
1971-1974	Northwestern University Medical School, Chicago, IL	M.D. (Honors Program in Medical Education)
Postgraduate		
1975	M. D. Anderson Tumor Institute Houston, TX	Jr Medical Fellow, Surgery
1975-1976	Strong Memorial Hospital Rochester, NY	Intern/Resident, Surgery
1976-1977	Strong Memorial Hospital Rochester, NY	Assistant Resident, Surgery
1978-1980	National Cancer Institute Bethesda, MD	Staff Fellow, Surgery Branch
1980-1982	University of Rochester	Sr. & Chief Resident, Surgery

Rochester, NY

2002	University of Pittsburgh Pittsburgh, PA	Mini-MBA Business Essentials for the Bio-Scientist
2002	ProteinChip University	Ciphergen ProteinChip Technology Protein Profiling Advanced Course; July 26, 2002

APPOINTMENTS AND POSITIONS

Academic

1972	University of Muenster, Westphalen West Germany	Res. Asst. Physiologische Institut II Biochemistry Bovine Fibrinogen
1975	Twelve Oaks Hospital Houston, TX	Emergency Room Physician
1980-1982	University of Rochester	Instructor in Surgery
1983-1988	Uniformed Services University Bethesda, MD	Assistant Professor of Health Sciences
1988-Present	Uniformed Services University Of the Health Sciences, Bethesda, MD	Associate Professor of Surgery
1990-Present	University of Pittsburgh Pittsburgh, PA	Professor of Surgery, Molecular Genetics and Biochemistry;
1990-2000		Chief, Section of Surgical Oncology; made a Division in 1998
1991-2000	Pittsburgh Genetics Institute Pittsburgh, PA	Codirector, Human Gene Therapy Program
1992-2000	Pittsburgh Cancer Institute Pittsburgh, PA	Codirector, Division of Biological Therapeutics
1995-Current	Pittsburgh Biotechnology, Inc	CEO and President
1999-2001	SmithKline Beecham Pharmaceuticals	Vice President and Director, Division of Inflammation, Tissue Repair & Oncology World Wide Discovery Biology; Research and Development
2001	GlaxoSmithKline Pharmaceuticals	Vice President and Director,

		High Throughput Biology; Discovery Research Biology; Research and Development
2002	Metacine, Incorporated	Chief Scientific Officer, Cofounder Sr. VP for Medical Affairs
2002-2006	University of Pittsburgh Molecular Medicine Institute	Director, Translational Research
2002	University of Pittsburgh School of Engineering, Dept Bioengineering	Professor of Bioengineering
2004	University of Pittsburgh McGowen Institute for Regenerative Medicine	Member
2006	Department of Surgery	Vice-chairman for Research Professor and Chief, Division of Translational Research
2006	University of Pittsburgh School of Medicine	Assistant Vice Chancellor, Interdisciplinary Research and Training

GOVERNMENT

1977-1978	National Health Service Corps Onamia, MN	Medical Officer
1982-1990	National Cancer Institute Bethesda, MD	Senior Investigator, Surgery Branch

CERTIFICATION AND LICENSURE

Speciality Certification

1983	American Board of Surgery	#29138 Surgery
1993	American Board of Surgery, Recertification	#29138 Surgery

MEDICAL OR OTHER PROFESSIONAL LICENSURE

1975	National Board of Medical Examiners	Diplomate
1976	New York	129152
1977	Minnesota	23584
1979	Maryland	D23864
1990	Pennsylvania	MD-042025-L

MEMBERSHIP IN PROFESSIONAL AND SCIENTIFIC SOCIETIES

American College of Surgeons, Fellow
American Medical Association
American Association of Cancer Research
- 1991 Program Committee

American Association of Immunology
- 1990, 1992 Program Committee
- 1996/97 Comoderator Tumor Immunology Minisymposium
- 1997-2000 Tumor Immunology Block Cochair

American Society of Clinical Oncology
- 1996/97 Program Committee
- 2004/2005 Biologic Therapeutics Program Committee
- 2005-2008 Grants Selection Committee

American Surgical Association
Association for Academic Surgery
Cell Transplant Society
Central Surgical Association
Clinical Immunology Society
- 1993 Program Committee
- 2001-2006 Federation of Clinical Immunology Societies, SBT Representative
- 2005-Present Executive Council

European Society of Tumor Immunology and Immunotherapy
International Society for Biologic Therapy of Cancer
- 1991-1993 Program Committee
- 1994-Present Executive Council
- 1996-1998 Vice President and President Elect
- 1998-2000 President
- 2003 Chair, Cancer Biometrics Workshop
- 2004 Chair, Cancer Biometrics Session
- 2004-Present Collaboration Committee

Melanoma Research Foundation
- 1996-Present Board of Directors

Molecular Medicine Society
Society for Analytical Cytology
Society of Biologic Screening, 2001
Society of Innate Immunity, 2006
Society of Surgical Oncology
- 1992,1993 Program Committee; 1993-1997 Clinical Affairs Committee
- 2000-2003 Research Award Committee
- 2005 Chair, Cancer Biometrics

Society of University Surgeons
Surgical Biology Club

EDITORIAL BOARDS

1988 European Cytokine Journal
1990 J. Immunotherapy (Formerly J. Biological Response Modifiers, Assoc. Editor)
1990-1995 J. Immunology
1990-1994 Contemporary Oncology
1991 General Surgery & Laparoscopy News (Surgical Oncology)
1991 Melanoma Research
1993 Therapeutic Immunology
1993 Cancer Research, Therapy and Control
1994 Cancer Gene Therapy, Associate Editor
1995 Clinical Cancer Research (Associated with AACR)
1995 Natural Immunity
1995 The Cancer Journal, Associate Editor
1995 Gene Therapy (Nature)
1995 Cytokines and Molecular Therapy
1996 Human Gene Therapy
1997 Cancer Therapeutics
1999–2001 Clinical Immunology
2000 Current Opinion in Investigational Drugs, Gene Therapy/Oncology Section Editor
2003 Journal Immunotherapy, Associate Editor
2003 The Oncologist, Associate Editor for Immunotherapy and Antibodies
2004 Molecular Cancer Therapeutics, AACR
2005 Translational Research

ACTIVITIES

1983-1990	Hospital Infection Control Committee, NIH
1984	Member, Source Evaluation Group RFP NCI CM 37613-64; "Phase I/II Clinical Evaluation of BRMs for the Treatment of Cancer"
1985-1990	Project Officer, Cell Sorting Operation, Surgery Branch, NCI
1985	FDA License Committee, Interferon Licensure in Melanoma
1985	Member, Source Evaluation Group RFP:NCI. CM 37613-64; MAO 4,5:Phase I Clinical Trial of Cytotoxic Activated Lymphocytes/IL-2
1985	Amer. Coll. Surgeons Rep.; Center for Disease Control Task Force: Recommendations for Preventing Transmission of Infection with Human T-Lymphotrophic VirusType III-Lymphadenopathy-associated Virus During Normal Procedures.
1985-1987	Coordinator, NIH Melanoma Working Group
1985-1987	Review Board, United Cancer Council, Inc., Rochester, NY
1987	Chairperson, Minisymposium on In Vivo Effects of Cytokines; FASEB, Washington, D.C. 4/1/87
1987	Representative, NCI Melanoma Clinical Strategy Group
1988-1990	Surgery Br. Rep., NCI Investigational Review Board
1989	Cochairperson, Minisymposium on T-cells and the Treatment of Cancer, AACR, San Francisco, CA; 5/25/89
1990	Ad Hoc Reviewer, Experimental Immunology Study Section, NIH
1990	Cancer Group, National Disease Research Interchange; 9/17/90
1990	Surgical Forum Moderator (Tumor Immunology), American College of Surgeons; San Francisco, CA; 10/8/90.
1990	Chairman, Special Study Section, Experimental Immunology, NIH
1990	Cancer Vaccine Workshop, NCI; Bethesda, MD; 10/29/90

1991	Planning Committee, NIH Consensus Development Conference on the Diagnosis and Treatment of Early Melanoma
1991	Participant, NIH Training Grant, University of Pittsburgh, "Molecular Mechanisms and Therapy of Childhood Diseases"
1991	Chairperson, Minisymposium on Antitumor Effectors; FASEB,Washington DC; 4/22/91
1991	Society of Surgical Oncology/Program Committee/Research and Government Relations Committee
1992 - 1995	American Society of Clinical Oncology Young Investigator Award, Clinical Development Award Committee
1992	Search Committee, Chair UPMC Director Radiation Oncology
1992-1995	External Advisory Committee, "Immunity to Lung Tumors and Melanoma by Gene Transfer to Tumors and Tumor-specific CTL", University of Miami Cancer Institute, PI, Eckhard R. Podack, MD
1993	What's New in Surgical Oncology, Amer. College of Surgeons
1993	Search Committee, Breast Cancer Center Director, UPMC/PCI
1993-2000	Mellon/Dickson Prize Committee; University of Pittsburgh School of Medicine; Chair 1997-2000
1994	Search Committee, NSABP Chairman, UPMC
1994	External Advisory Committee, "Gene Therapy for Solid Tumors", Baylor College of Medicine, PI, Savio Woo, Ph.D.
1994-Present	Executive Council, Society for Biologic Therapy; Vice President (1996); President 1998-2000; Immediate Past President 2000-2002.
1995-1996	UICC/American Cancer Society Fellowship Committee
1996	External Advisory Committee, "Gene Therapy of Cancer", Memorial Sloan-Kettering Cancer Institute, PI, Lucio Luzzatto, MD
1997	Site Visit Committee, National Institutes of Health, National Cancer Institute; Frederick Cancer Research Center, Branch Chief, John Ortaldo.

1997 UPMC/Health Sciences International Committee

1997 Site Visit Committee, National Institutes of Health, National Cancer Institute; Section of Tumor Immunology, Branch Chief, Jeffrey Schlom.

1997	Site Visit Committee, National Institutes of Health, National Cancer Institute; 1RO1 CA76489-01 Dr. Donald L. Morton Polyvalent Vaccine: Phase III Trial in Stage IV Melanoma; John Wayne Cancer Institute; August 5, 1997.
1997-2000	Cardinal Bernardin Cancer Center External Advisory Council (Loyola University Medical Center)
1997-2003	Melanoma Research Foundation, Board of Trustees
1998	TK Immunotherapy Expert Panel; Rhone Polenc Rorer
1998	Search Committee, Chief of Urology University of Pittsburgh
1998-2001	Ohio State University Scientific Advisory Committee; Arthur G. James Cancer hospital and Research Institute
1998	Biosafety Committee, Mercy Hospital (Adenoviral p53 Gene Therapy)
1998	Clinical Trials Steering Committee; University of Pittsburgh Medical Center
1998	Search Committee, Chief of Medicine University of Pittsburgh
1998	Standing Committee for Faculty Recognition and Research Awards, U. Pittsburgh
1997-2001	Peer Review Committee on Cliical Research, Cancer Control and Epidemiology; American Cancer Society
1999	Co-Director, Gene Therapy Program, SmithKline Beecham
1999	Co-Director, Protein Agent Strategic Initiative, SmithKline Beecham
1999-2000	Co-chair, Cancer Gene Therapy Committee of the American Society of Gene Therapy (ASGT)
2000	Sponsored, developed, and ran SB Symposium on "Tissue Repair and Wound Healing, Therapeutic Opportunities" Upper Providence, March 2-3, 2000.
2000	Grand Rounds, University of Pennsylvania Cancer Center; In Vivo Veritas – Lessons for Immunotherapy of Cancer; April 20, 2000.
2001	New York Academy of Sciences; Codirector; Symposium on Use of Viral Vectors for Target Validation with Tom Kost; December 4, 2001
2001	Study Section; Chemoprevention of tobacco-related cancers in former smokers:

preclinical studies; RFA CA-02-008; December 6, 2001; Nov 4, 2002.

2002 American Society of Clinical Oncology Discussant; Annual Meeting, Orlando; Enhancing the antitumor effects of IL-2.

2002 Amer. Association for Gene Therapy; Meet the Professor Session – Immunology

2002 James Ewing Young Investigator Award for Clinical Research; Society of Surgical Oncology; Selection Committee

2002 External Advisor/ PI Savio Woo Mt. Sinai School of Medicine; Gene Therapy of Colorectal Cancer

2002 Scientific Advisory Board, Association for Cancer Gene Therapy

2002 Guest Editor with Brigitte Autran, Hervé Fridman, and Bruce Walker. Special Issue – Vaccine [Volume 20 Supplement 4 19 December 2002]: Therapeutic Vaccines Against HIV and Cancers 23-26 June 2002, Les Pensières, Veyrier-du-Lac, Annecy, France; Organized by the Merieux Foundation with the support of Aventis Pasteur and Mérial

---

2003 External Advisor/PI Thomas Kupper Harvard Medical School; NCI funded Melanoma SPORE

2003 Coorganizer with Laurence Zitvogel: FOCiS-iSBTc Satellite Symposium on Cellular Immunology and the Immunotherapy of Cancer V Thursday Afternoon May 15th, 2003

2003 Liver Proteomics Meeting, NIDDK; Chaired by Laura Beretta

2003 Esternal Advisor/PI Hans-Georg Klingermann Rush-Presbyterian St. Luke's Medical Center; Immunotherapy for Bone Marrow Grafting

2003 Coorganizer 1st International Society of Biological Therapy Workshop on Proteomics, Genomics and High Content Cellular Screening in Patients with Cancer with Ena Wang, MD, PhD Dept. of Transfusion Medicine, National Institutes of Health; Nabil Hanna, PhD, Chief Scientific Officer, IDEC Pharmaceuticals Masur Auditorium, National Institutes of Health; October 30, 2003

2003 External Advisor/Consultant; Immune Tolerance Network

2004 Grand Rounds, Roswell Park Cancer Institute; January 21, 2004

2004	Symp. VIII Moderator, Biology and Therapeutic Application of DCs in Chronic Inflammation; 6th Congress - Trauma, Shock, Inflammation and Sepsis; March 3, 2004
2004-2005	NCI Clinical Oncology Study Section June, October, February [Ad hoc]
2004-2005	AACR/ASCO Vail Clinical Protocol Development Faculty
2004-2006	Scientific Programs Committee ASCO
2004	NCI Study Section, Strategic Partnering to Evaluate Cancer Signatures [RFA-CA-04-015]; November 18-19, 2004
2005	ASCO Member of the Program Committee; Developmental Therapeutics: Immunotherapy Track
2005, 2006	Mentor; The Jack Kent Cooke Foundation and the American Psychological Association's Center for Gifted Education Policy; APEX Program
2005	NCI Study Section, Metabolism Branch, Branch Chief Thomas Waldmann
2005	NIH GCRC Study Section, University of California at Los Angeles; 12/05/05
2006	Liver Center and Liver Cancer PO1 Advisor, A. DiBesceglie, St. Louis University
2006	External Advisory Board for the Human Immunology Center at the University of Rochester Medical Center in Rochester, New York; PI Tim Mosman

Honors

1971-1974	Honors Program in Medical Education
1982	Robert H. White Award for Excellence in Teaching, Univ. of Rochester
1986	Edith Hamilton Cancer Lecturer, Genesee Hospital/Wasyl Pluta Cancer Center, Rochester, NY
1987	Special Achievement Award; Dept. Health & Human Services, NIH.
1988	4th Vender Lecturer, Northwestern University; Evanston, Illinois
1989	Visiting Professor, Dept. of Surgery, Duke University; Durham, NC
1990	Coorganizer Keystone Symposium on Cellular Immunity and the Immunotherapy of Cancer, Park City, UT
1990	Virginia Mason Res. Center Distinquished Lecturer, Seattle WA
1990	14th Annual Lecturer, Internal Medicine Group; New Orleans, LA.
1991	Annual John Palmer Lecturer, Univ. of Toronto; Toronto, Ontario
1991	Tenth Hinshaw Lecturer, Univ. of Rochester, Rochester, New York
1992	Coorg. Keystone Symp.on Melanoma and Biology of the Neural Crest, Taos, NM
1992	Coorganizer, Roundtable Roussel-UCLAF on Cytokines and Cancer, Versailles, France, 10/7-9/92
1992	Chairman, Cancer Care Committee, Pittsburgh Cancer Institute
1993	Organizer, Symposium Molecules to Medicine, 2nd Inter. Congress on Biol. Response Modifiers; San Diego, CA; 1/29/93
1993	Coorganizer Keystone Symposium on Cellular Immunity and the Immunotherapy of Cancer II, Taos, NM; 3/17-24/93.
1993	Cochairman, Symp.Tumor Immunology, Denver CO; AAI/CIS 5/29/93.
1993	Visiting Professor of Surgical Oncology; Academia Sinica/Veteran's General Hospital; Taipei, Taiwan; 6/27/93-7/15/93.

1994	NIH Committee (Mail Ballot-Scientific Meetings and Conferences).
1994	Sommer Memorial Lecture, Portland, Oregon; April 21-22, 1994.
1994	Site Visit Chairman, NIH PO1-CA64254-01. Brain Tumor Gene Therapy; June 14-16, 1994
1994	Plenary Speaker, Centennial Celebration of the Royal Victoria Hospital; June 9, 1994
1995	EJ Tabah Lectureship, McGill University; March 13-15, 1995.
1995	First Peter Finke Lecturer; Memorial Sloan Kettering; Sept. 15, 1995.
1995	Coorganizer, New York Academy of Sciences Symposium: Interleukin 12; An important regulatory cytokine. November 9-12, 1995; NYC.
1996	Reverse Site Visit Committee Stanford University Medical Center General Clinical Research Center; July 30, 1996; Rockville, MD.
1996	Site Visit Committee; Laboratory of Experimental Immunology; Div. of Basic Sciences; NCI; Frederick, MD; November 5, 1996.
1996	McCutcheon Lectureship, University of Toronto; November 8, 1996.
1997	Distinguished Lecturer, Robert Wood Johnson UMDNJ; January 15, 1997.
1997	Coorganizer, Keystone Symposium on Cellular Immunology and the Immunotherapy of Cancer; Copper Mountain, February 1-7, 1997.
1997	Distinguished Lecturer, Westmoreland Hospital; Greensberg, PA
1997	University of Pittsburgh Cancer Institute Scientific Leadership Award
1998	Coorganizer, 5th International DC Meeting; Pittsburgh; September 24-28, 1998.
1998	Coorganizer, 13th Society for Biological Therapy Meeting, Pittsburgh; October 21-21, 1998.
1998-2001	Visiting Professor of Oncology, Shanghai Medical University (SMU)
2000	Co-Organizer, 4th Keystone Symposium on Cellular Immunology and the Immunotherapy of Cancer; Santa Fe, NM January 21-27, 2000

2000	Keynote Speaker, American Association of Cancer Research Special Meeting on Melanoma; The Woodlands, Texas May 3-7, 2000.
2000	Coorganizer, American Association of Cancer Research Special Meeting on Cytokines; Vail Colorado; September 20-23, 2000.
2000	Visiting Professor; Wistar Instititute; Philadelphia, PA; April 28, 2000.
2001	Plenary Lecture, Japanese Surgical Society, April 2001
2002	Danny Hill Tumor Immunology Lectureship, University of Western Australia, Perth, Australia
2002	Surgical Grand Rounds, Columbia University August 29, 2002.
2002	Surgical Grand Rounds, Montefiore Hospital/Albert Einstein University; October 21, 2002.
2003	Surgical Forum; Plenary Speaker Society Surgical Oncology; March 10, 2002. Los Angeles, CA
2003	Keynote Speaker Carolyn Frye-Halloran Symposium and Grand Rounds Combined Neurology, Neurosurgery, Psychiatry Grand Rounds; Massachussetts General Hospital, September 04, 2003; Boston, MA.
2003	Surgical Grand Rounds, Northwestern University/Evanston Hospital; October 10, 2003
2004	Organizer Biology and Therapeutic Application of DCs In Chronic Inflammation 6th World Congress on Trauma, Shock, Inflammation and Sepsis – Patho-physiology, Immune Consequences and Therapy March, 2004, Munich
2004	Dermatology Grand Rounds, Johns Hopkins University; June 16, 2004.
2004	Member Ad Hoc Scientific Counselors, Clinical Center NIH [Review Dept Transfusion Medicine]
2004, 2005	Ad Hoc Member, Clinical Oncology Study Section, NCI
2005	Member NCI Review Committee, Metabolism Branch, NCI
2005	Hillman Award Recipient, UPCI
2006-2009	Member, Clinical Oncology Study Section, NCI
2006	Coorganizer, SRI Innate Immunity World Congress

REGULATORY EXPERIENCE WITH FDA/RAC/IRBS

1979	TCGF Expanded Lymphocytes for Cancer [Clinical Center, NIH]
1981	^{111}In oxine labeled leucocytes; personnel IND in setting of transplantation
1983	Jurkat-derived IL-2; Personal IND and clinical protocol with Dupont
1985	Recombinant IL-2; Intraperitoneal administration; Cetus – IND holder; NCI protocol
1987	Recombinant IL-4; IND with FDA to MTL alone/combination with IL-2; Sterling Pharmaceuticals
1989	Recombinant IL-2 and Immunoglobulins; IND with FDA to MTL; with Cetus
	Presentation to RAC on Neo-transfected TILs for cancer therapy
1991	IND IL-2 and activated natural killer cells for regional infusion
1992	IL-4 Gene Therapy; RAC/FDA IND/IRB submissions
1995	IL-12 Gene Therapy; RAC/FDA IND/IRB submissions
	Presentation to FDA on rIL-2 for Chiron for licensing in melanoma
1998	DC based therapy of melanoma; FDA IND/IRB submissions
2001	Pre-IND and IND meetings for GlaxoSmithKline on IL-18 protein therapy
2003	Pre-IND meetings for iNOS gene therapy for vascularized shunts
2003	Integrating NK and DC in the therapy of cancer; protocol development; IND preparation
2004	Protocol development, combined biochemotherapy of melanoma

Research Grants

9/92-10/96	NIH 1UO1 CA 58272-01 "Locoregional ALT with autologous IL-2, activated NK cells", Principal Investigator, $800,000.
9/92-10/94	NIH 1PO1 CA59371-01 "Gene Therapy of Cancer - Immunological Approaches", Principal Investigator, $750,000.
9/92-8/94	NIH 1ROA CA56088-01A1 "Specific T-cell Recognition of Human Melanoma", Principal Investigator, $100,000.
8/93-9/97	NIH 1RO1 CA 57804-01A1 "Identification of Class I Presented Peptides", Co-Principal Investigator, $466,808.
10/91-10/93	VA RAG "Breaking Tolerance with Cytokine Based Cancer Immunotherapy", Coinvestigator, $68,000.
1995-1998	Schering Plough Research Institute. "Gene Therapy with IL-4, IL-10, vIL-10, and interferon alpha", Principal Investigator, $1,000,000.
4/96-5/01	NIH (1RO1CA63350-01) "Dendritic Cell Based Therapies Designed for Murine Tumors", Co-Principal Investigator, $1,060,434.
2/94-3/99	NIH (NCI-CM-47001-64) "Clinical Trials of Biologic Response Modifiers", Principal Investigator, $2,874,341.
4/94-3/96	NIH (NCI-1R21CA69106-01) "Emulsion Based Therapy of Cancer", Coinvestigator, $96,000.
7/95-6/99	NIH (NCI-1P01 CA 68067-01) "Cytokine Gene Therapy of Cancer", Principal Investigator, $4,261,226.
11/95	NIH (1 R13 CA68006-01) "Interleukin 12: Immunology of a Regulatory Cytokine",Principal Investigator, $47,900.
10/96	NIH 1PO1 DE12321. "Vaccine Development for Head and Neck Cancer," Principal Investigator, Project 3. PI Dr. Theresa Whiteside, $2,500,000.
8/96-6/00	NIH NCI 1UO1CA74329-01. "Clinical Trials of Biological Response Modifiers. Principal Investigator, $550.951.
02/97	NIH 1R13CA73576-01. "Conference on Cellular Immunology of Cancer," Principal Investigator, $50,000.

04/97-03/02	NIH NCI-1RO1CA73816-01."Dendritic cells elicit effective antitumor responses," Coinvestigator (PI, Walter J. Storkus), $1,429,008.
7/97-6/02	NIH NCI 1PO1DE12321-01. "Vaccine Development for Oral Carcinoma." Principal Investigator, Project 1. PI Dr. Theresa Whiteside, $2,600,000
11/97	NIH NCI-1PO1CA73743-01. "DC Biology and Therapy", CoPI, $9,978,000.
11/97	NCI-1K12CA76906-01. "Biologic Therapy Research Career Development Program." Co Principal Investigator, $1,660,371.
1998	Argonex, Inc. "Identification of T-cell targets in colorectal and ovarian carcinoma." Principal Investigator, $137,952.
01/98-12/02	NIH NCI 1PO1CA7374301A1 "Dendritic Cell Biology and Therapy. Principal Investigator, $9,021,156.
09/98-09/02	PAR-97-080 "Novel HIV Therapies: Integrated Preclinical/Clinical Program"; Project 4: Dendritic Cell Therapy for HIV: Role of Cytokines on Enhanced T-cell Function"; Total $1,380,101; PI Michael Lotze, CoPI Cara Wilson.
06/01/99-05/30/04	"Research Training in AIDS, STDs and Emerging Infections;" PI David Tweardy; Total $747,573; Mentor/Training Faculty
03/00-02/05	NIH NCI 1RO1CA82016-01A29 "Melanoma Associated T & DC Dysfunction and Death. Principal Investigator, $1,676,406.
04/00-03/05	NIH NCI 2PO1CA68067 Cytokine Gene Therapy of Cancer, CoPI, $9,861.090
7/8/05 – 6/30/10	1 PO1 CA 101944-01A2 (Lotze, Michael T) Integrating NK and DC into Cancer Therapy National Cancer Institute $11,000,064 60%
3/1/04-2/29/08	1 RO1 CA 100415-01 $1, 189,733; Development of a tumor selective replicating vaccinia; David L. Bartlett, PI; MTL, coinvestigator 10%
04/01/04	RFA-RM-04-005 National Technology Centers for Networks and Pathways Fluorescent Probes and Imaging for Pathways; Alan S. Waggoner, PI; National Institutes of Health; $14,435,640; Michael T. Lotze Co-PI; 30%
06/01/04	1PO1 CA 113698-01; Regional Therapy of Cancer; Submitted to the NCI; "3 projects; 3 cores; Principal Investigator, CoPI, David Bartlett; $10M EDRN
04/01/04	Project 2: Luminex Multianalyte and SELDI-TOF-MS Serum Proteomic Profiling of Pancreatic Cancer; Herbert J. Zeh, PI; Michael T. Lotze, 10%

07/01/04	1RO1CA106534-01 Detection of Molecular Markers of Melanoma and Response to IFN Therapy; CoPI, JM Kirkwood, MT Lotze, WL Bigbee; 10%; $1,850,000
09/01/04	Tolerogenic DC Therapy for Thoracic Organ Transplant. PI Kenneth R. McCurry; Coinvestigator, Michael Lotze; 5% + technical support 50%; $1,843,745
10/01/04	NIDDK; Chronic Inflammation of the Liver. PI Anthony J. Demetris; Co-PI, Michael T. Lotze; 30%; $5,000,000
10/01/05-9/30/10	NIDDK; Gut Inflammation. PI Michael T. Lotze; Co-PI, Mitchell Fink; 30%; $5,000,000
12/1/05-11/30/07	1 R21 CA115059-01A1 (Bartlett, David L) $750,000 10% MTL and 10% Richard DeMarco Isolated Hepatic Perfusion with Oxaliplatin
4/01/06-3/30/11	AP4 Proposal to NCI [Lilly, UPCI, Cellumen/Pittsburgh Life Sciences Greenhouse] Ronald B Herberman CoPI, Michael T. Lotze 40% $6,750,000 Submitted
4/01/06	Shared Instumentation Grant to the NCRR, $532,000 VTI Cellomics ArrayScan Cytometer
4/15/06	Interdisciplinary Research Proposal to NIH

PATENTS

Storkus Walter, Michael T. Lotze. Rapid Isolation of T-cells Epitopes from viable cells by mild acid elusion. (November 23, 1999; 5,989,565)

Baar Joseph, Michael T. Lotze. Interferon/gamma inducible cytokine expression plasmids.

Tahara Hideaki, Michael T. Lotze. *In situ* injection of antigen-presenting cells with genetically enhanced cytokine expression. U Pittsburgh Reference Number 181; 09/395,836. Filed September 14, 1999; issued August 6, 2002.

Thomson Angus, Lu Lina, Michael T. Lotze - "Genetic engineering of dendritic cells for immunosuppressive therapy" Disclosure

Tahara Hideaki, Michael T. Lotze. Modified interferon gamma inducing factor (IGIF/IL-18) sequence which can be secreted as an active form IL-18 protein from mammalian cells.

Angus W. Thomson, Lina Lu, Michael T. Lotze. Genetically Modified Antigen Presenting Cells for the Induction of Immunointolerance.

Siamak Agha-Mohammadi, Michael T. Lotze. PCT/US01/31138. High Efficiency-Regulatable Expression System

FILMS

1986 Demonstration of intraoperative ultrasound imaging, CO_2 laser surgery and CUSA ultrasonic dissection for a right hepatic lobectomy for hepatoma; Spectacular Problems in Surgery, ACS

1992 Immunotherapy Video Handbook, Proleukin^R

1995 Resection of a Giant Lipoma; ACS Cine Forum, New Orleans, October 22-27, 1995.

BOOKS

1990 Cellular Immunity and the Immunotherapy of Cancer, Ed. Lotze MT, Finn OJ, Wiley-Liss; New York, 1990.

1994 Current Cancer Therapy, Ed. Kirkwood JM, Lotze MT, and Yasko J; 1998 Current
1996 Science, Philadelphia, 1994; 2nd Edition 1996; 3rd Edition 1998; 4th Edition 2001.
1998
2001

1996 Interleukin 12: Cellular and Molecular Immunology of an Important Regulatory Cytokine. Ed. Michael T. Lotze, New York Academy of Sciences, NY.

1997 Regional Therapy of Advanced Cancer, Ed. Lotze MT, Rubin JT; JB Lippincott, Philadelphia, 1997.

1999 Dendritic Cells: Biology and Clinical Applications; Ed. Michael T. Lotze, Angus W.
2001 Thomson; Academic Press; London; 2nd Edition 2001
2006 3rd Edition; Ed. Michael T. Lotze, Jacques Banchereau, Yong-Jun Liu, and Angus W. Thomson

2002 Tumor Immunology: Molecularly Defined Antigens and Clinical Applications. Ed. Giorgio Parmiani and Michael T. Lotze; Taylor and Francis, London; 2002.

2003 Cytokine Handbook, 4th Edition. Ed. Angus W. Thomson, Michael T. Lotze; Academic Press, London; 2003.

2005 Measuring Immunity, 1st Edition. Ed. Michael T. Lotze, Angus W. Thomson; Academic Press, London.

2007 Natural Killer Cells, 1st Edition. Ed. Michael T. Lotze, Angus W. Thomson; Academic Press, London.

SCIENTIFIC ADVISORY BOARDS

1990-1994	Cellco, Inc. Rockville, MD; Hollow-fiber growth of cells
1995-1998	Canji, Inc; Subsidiary of Schering Plough, Inc. Gene Therapy [p53, RB, cytokines]
2002-current	Immunodesigned Molecules, Inc. Parisian company involved with cell/DC therapy
2002-current	Tissue Informatics, Inc. Pittsburgh company involved with machine vision tissues
2002-current	CureTech, Inc. Tel Aviv company involved with developing antibody therapies
2002	BioMeasure; Boston; consultant on therapeutic vaccines for cancer
2003	MediGene, Munich; consultant on therapeutic vaccines for cancer
2003	Coley Pharmaceuticals, Wellesley; consultant on biomarkers/surrogates for cancer
2003	Allergan Pharmaceuticals, Irvine, CA; consultant on immunogeneicity of recombinant proteins
2003-Present	Medarex Pharmaceuticals/Bristol Myers Squibb, Bloomsfield NJ; consultant on clinical trials with MDX-010
2004	ZelleRx, Chicago, IL; consultant on therapeutic use of NK cells; on Scientific Advisory Board
2004	Becton Dickinson; consultant on antibody development and application in flow and imaging cytometry
2004	Chiron; consultant on development of combination therapies with IL-2
2005	Sanofi-Aventis; consultant on cancer vaccines
2005	Debios, Lausanne

RACES/MARATHONS

1985	Marine Corps
1990	Pittsburgh
1993	Pittsburgh
1998	Pittsburgh, Bordeaux [Marathon des Chateaux du Medoc], Marine Corps
1999	Pittsburgh, Cincinnati Flying Pig, Helsinki, San Diego Rock and Roll, New York
2000	Philadelphia, Boulder Outback, Quebec City, Marine Corps, Vancouver, Jacksonville
2001	Philadelphia, Pittsburgh, London, Seattle
2002	Paris, Pittsburgh, Buffalo, San Francisco, Bordeaux [Marathon des Chateaux du Medoc], New York [1/2], Seattle
2003	Chicago, Marine Corps
2004	Phoenix Rock and Roll, IKEA Half, New York Half, Tucson Half
2005	Nashville, Utica Boilermaker 10M, Presque Isle Half, Akron, Marine Corps Marathon, Las Vegas Marathon
2006	IKEA Half
2007	Los Angeles, Cleveland, San Diego Rock and Roll

BIBLIOGRAPHY

1. Lotze, M.T., Strausser, J.L., Rosenberg, S.A. *In vitro* growth of cytotoxic human lymphocytes. II. Use of T cell growth factor (TCGF) to clone human T cells. J. Immunol. 124:2972-2978, 1980.

2. Dubois, M., Lotze, M.T., Diamond, W.J., Kim, Y.O., Flye, W.M., Macnamara, T.E. Pulmonary shunting during leukagglutinin induced noncardiac pulmonary edema. JAMA 244:2186-9, 1980.

3. Lotze, M.T., Lines, B.T., Strausser, J.L., Rosenberg, S.A. Tumor lysis by human T lymphocytes in long term culture and their distribution *in vivo*: Implications for immunotherapy. Surg. Forum 31:404-406, 1980.

4. Lotze, M.T., Lines, B.T., Mathisen, D.J., Rosenberg, S.A.: The in vivo distribution of cultured autologous human and murine lymphoid cells grown in T cell growth factor (TCGF): Implications for the adoptive immunotherapy of tumors. J. Immunol. 125:2972-2978, 1980.

5. Bias, W.B., Hsu, S., Pollard, M.K., Harvey, J., Lotze, M.T., Arnett, F.C., Stevens, M.B.: HLA-DR characterization of a Chippewa Indian subpopulation with high prevalence of rheumatoid arthritis. Human Immunol. 2:155-163, 1981.

6. Lotze, M.T., Rosenberg, S.A.: *In vitro* growth of cytotoxic human lymphocytes. III. The preparation of lectin free T cell growth factor (TCGF) and an analysis of its activity. J. Immunol. 126:2215-2220, 1981.

7. Lotze, M.T., Grimm, E.A., Mazumder, A., Strausser, J.L., Rosenberg, S.A.: Lysis of fresh and cultured autologous tumor by lymphocytes cultured in T cell growth factor (TCGF). Cancer Res. 41:4420-4425, 1981.

8. Lotze, M.T., Duncan, M.A., Gerber, L.H., Woltering, E.A., Rosenberg, S.A.: Early versus delayed motion following axillary dissection: A randomized, prospective study. Ann. Surg. 193: 288-295, 1981.

9. Harvey, J., Lotze, M.T., Stevens, M.B.: Rheumatoid arthritis in a Chippewa band. I. Pilot screening study of disease prevalence. Arth. Rheum., 24: 717-721, 1981.

10. Strausser, J.L., Mazumder, A., Grimm, E.A., Lotze, M.T., Rosenberg, S.A.: Lysis of human solid tumors by autologous cells sensitized *in vitro* to alloantigens. J. Immunol. 127: 266-271, 1981.

11. Rosenberg, S.A., Grimm, E.A., Lotze, M.T., Mazumder, A.: The growth of human lymphocytes in T-cell growth factor: Potential applications to tumor immunotherapy. Lymphokines. Mizel, S.F. (ed), Academic Press: New York, 1982.

12. Rosenberg, S.A., Eberlein, T.J., Grimm, E.A., Lotze, M.T., Mazumder, A., Rosenstein, M.:

Development of long-term cell lines and lymphoid clones reactive against murine and human tumors: A new approach to the adoptive immunotherapy of cancer. Surgery 92:328-335, 1982.

13. Harvey, J., Lotze, M.T., Bias, W.B., Hsu, S., Arnett, F.C., Stevens, M.B.: Rheumatoid arthritis in a Chippewa band. II. Field study with clinical, serologic and HLA-D correlations. J. Rheumatology. 10:28-32, 1983.

14. Lotze, M.T., Marquis, D.M., Carey, A.S., Byer, B.J., Hoy, W.E., May, A.G.: Two new assays for the early detection of transplant rejection, Interleukin-2 response and PHA-augmented NK activity. Transplantation Proceedings 15:1796-1799, 1983.

15. Duncan, M.A., Lotze, M.T., Gerber, L., Rosenberg, S.A.: Incidence and recovery of serratus anterior palsy following axillary dissection. J Amer. Phys. Ther. Assn. 63:1243-1247, 1983.

16. Ouriel, K., Lotze, M.T., Hinshaw, J.R.: Prognostic factors of carcinoma of the male breast. Surg. Gynec. Obst. 159:373-376, 1984.

17. Donohue, J.H., Rosenstein, M., Chang, A.E., Lotze, M.T., Robb, R.J., Rosenberg, S.A.: The systemic administration of purified Interleukin-2 enhances the ability of sensitized murine lymphocyte lines to cure a disseminated syngeneic lymphoma. J. Immunol. 132:2123-2128, 1984.

18. Grimm, E.A., Vose, B.M., Chu, E.W., Wilson, D.J., Lotze, M.T., Rayner, A.A., Rosenberg, S.A.: The human mixed lymphocyte tumor cell interaction test I. Positive autologous lymphocyte proliferative responses can be stimulated by tumor cells as well as by cells from normal tissues. Cancer Immunol. & Immunotherapy 17:83-89, 1984.

19. Donohue, J.H., Lotze, M.T., Robb, R.J., Rosenstein, M., Braziel, R.M., Jaffe, E.S., Rosenberg, S.A.: *In vivo* administration of purified Jurkat derived Interleukin-2 in mice. Cancer Research 44:1380-1386, 1984.

20. Rosenberg SA, Rosenstein M, Grimm E, Lotze, M, Mazumder A. The use of lymphoid cells expanded in IL-2 for the adoptive immunotherapy of murine and human tumors.In Thymic Hormones and Lymphokines. Goldstein A.L.; Plenum Pub. Co., NY, 1984; pgs. 191-207.

21. Chang AE, Lotze MT, Ames RS, Rosenberg SA. A large scale method for the purification of Interleukin-2 secreted by the murine EL-4 thymoma. J. Immunopharmacology 7:17-31, 1985.

22. Lotze, M.T., Robb, R.J., Frana, L.W., Seipp, C.A., Sharrow, S.O., Gelman, E.T., Longo, D.L., Rosenberg, S.A.: Clinical studies with purified human IL-2 in patients with the acquired immunodeficiency syndrome and cancer in UCLA Symposium:AIDS; Alan R. Liss, Inc. Press, NY, 1984; J. Groopman, M. Gottlieb.

23. Lotze MT, Rosenberg S. *In vivo* use of cytokines.Clin. Immun. Newsletter, 5:116-120, 1984.

24. Lotze, M.T., Robb, R.J., Sharrow, S.O., Frana, L.W., Rosenberg, S.A.: Systemic administration of interleukin-2 in man. J. Biol. Resp. Mod. 1984, 3:475-482, 1984.

25. Lotze, M.T., Rosenberg, S.A.: Immunologic changes in patients with cancer given interleukin-2 (IL-2) in a phase I trial. Surg. Forum, 1984, 35:407-408, 1984.

26. Rayner, A.A., Grimm, E.A., Lotze, M.T., Rosenberg, S.A.: Demonstration of shared recognition and lysis of autologous and allogeneic fresh human tumors by cloned lymphokine activated killer (LAK) cells. Surg. Forum, 1984, 35: 415-417.

27. Lotze, M.T., Sugarbaker, P.H.: Femoral artery based myocutaneous flap for hemipelvectomy closure; Amputation following failed limb-sparing surgery and radiation therapy. Am. J. Surg. 150: 625-630, 1985.

28. Rayner, AA, Grimm, EA, Lotze, MT, Wilson, DJ, Rosenberg, SA: Lymphokine-activated killer (LAK) cell phenomenon:IV. Lysis by LAK cell clones of fresh human tumors from autologous and multiple allogeneic tumors. JNCI 75:67-75, 1985.

29. Rayner, A.A., Grimm, E.A., Lotze, M.T., Chu, E.W., Rosenberg, S.A.: Lymphokine activated killer (LAK) cells. Analysis of factors relevant to the immunotherapy of human cancer. Cancer 55:1327-1333, 1985.

30. Lotze MT, Frana LW, Sharrow SO, Robb RJ, Rosenberg SA. In vivo administration of purified human IL-2 I.Half life and immunologic effects of the Jurkat cell line derived Interleukin 2. J. Immunol. 134:157-166, 1985.

31. Lotze, M.T., Rosenberg, S.A.: Treatment of tumor patients with purified human interleukin-2. (In) Cellular and Molecular Biology of Lymphokines. Academic Press, 1985, pp. 711-721.

32. Lotze, M.T.: Current and future research directions in management of hepatic cancer in Hepatoma, ed. H. Wanebo, Marcel Decker, NY, 1986, pp. 501-534.

33. Lotze, M.T., Rayner, A.A., Grimm, E.A.: Problems with the isolation of lymphoid clones with reactivity to human tumors. Behring Institute Research Communications., 1985, 77:105-14.

34. Longo, D.L., Steis, R.G. , Lane, H.C., Lotze, M.T., Rosenberg, S.A., Preble, O., Masur, H., Rook, A.H., Fauci, A.S., Jacob, J., Gelmann, E.P.: Malignancies in the AIDS patient: Natural history, treatment strategies, and preliminary results. Ann. N.Y. Acad Science, 837:421-430, 1984.

35. Lotze, M.T., Rosenberg, S.A.: Treatment of immunologic disorders in AIDS patients. In Devita VT, Hellman S, Rosenberg SA (eds): Acquired Immune Deficiency Syndrome. Philadelphia, J.B. Lippincott, 1985: pp. 235-264.

36. Lotze, M.T., Matory, Y.L., Ettinghausen, S.E., Rayner, A.A., Sharrow, S.O., Seipp, C.A.Y.,

Custer, M.C., Rosenberg, S.A.: In vivo administration of purified human interleukin-2. II. Half life, immunologic effects and expansion of peripheral lymphoid cells in vivo with recombinant interleukin-2. J. Immunol. 135:2865-2875, 1985.

37. Lotze, M.T., Matory, Y.L., Rayner, A.A., Ettinghausen, S.E., Seipp, C.A., Rosenberg, S.A.: Toxicity of interleukin-2 in patients with cancer. Cancer. 58:2764-2772. 1986.

38. Skibber, J.M., Lotze, M.T., Garra, B., Fauci, A.: Successful management of hepatic abscesses by percutaneous catheter drainage in chronic granulomatous disease. Surgery. 99:626-630, 1986.

39. Lotze, M.T.: Stemming Cancer in the 80's. AIDS: A surgeon's responsibility. Bulletin of the American College of Surgeons. 70:6-12, 1985.

40. Edington, H., Lotze, M.T.: V-Y closure in abdominal wall stoma reduction. Surgery, Gynecology, and Obstetrics. 164:381-382, 1987.

41. Lotze, M.T., Custer, M.C., Sharrow, S.O., Rubin, L.A., Nelson, D.L., Rosenberg, S.A.: In vivo administration of purified human interleukin-2. III. Development of Tac positive cells and circulating soluble interleukin-2 receptors following interleukin-2 administration to humans. Cancer Res. 47:2188-2195, 1987.

42. Rosenberg, S.A., Lotze, M.T.: Cancer immunotherapy using interleukin-2 and interleukin-2-activated lymphocytes. Ann. Rev. Immunol. 4:681-709, 1986.

43. Rosenberg, S.A., Lotze, M.T., Muul, L.M., Leitman, S., Chang, A.E., Ettinghausen, S.E., Matory, Y.L., Skibber, J.M., Shiloni, E., Vetto, J.T., Seipp, C.A., Simpson, C., Reichert, C.M.: Observations on the systemic administration of autologous lymphokine activated killer cells and recombinant interleukin-2 to patients with metastatic cancer. NEJM. 313:1485-1492, 1985.

44. Perez, P., Titus, J.A., Lotze, M.T., Cuttita, F., Longo, D.L., Groves, E.S., Rabin, H., Durda, P.J., Segal,D.M.: Specific lysis of human tumor cells by T cells coated with anti-T3 crosslinked to anti-tumor antibody. J. Immunol. 137:2069-2072, 1986.

45. Rosenberg, S.A., Lotze, M.T., Muul, L.M., Leitman, S., Chang, A.E., Vetto, J.T., Seipp, C.A., Simpson, C.A.: A new approach to the therapy of cancer based on the systemic administration of autologous lymphokine activated killer cells and recombinant interleukin-2. Surgery 100:261-71, 1986.

46. Lotze, M.T., Chang, A.E., Seipp, C.A., Simpson, C., Vetto, J.T., Rosenberg, S.A.: High dose recombinant interleukin-2 in the treatment of patients with disseminated cancer: Responses, treatment related morbidity and histologic findings. JAMA. 256: 3117-3124, 1986.

47. Lotze, M.T., Rosenberg, S.A.: Results of clinical trials with the administration of interleukin-2 and adoptive immunotherapy with activated cells in patients with cancer. Immunobiology. 172: 420-437, 1986.

48. Lotze, M.T., Rosenberg, S.A.: Protocol design for lymphokine testing in clinical studies of human cancer. Lymphokine Res. 5: S177-S182, 1986.

49. Lotze, M.T, Custer, M.C., Rosenberg, S.A.: Intraperitoneal administration of interleukin-2 in patients with cancer. Arch. Surg. 121: 1373-1379, 1986.

50. Lotze, M.T.: Surgical considerations in the diagnosis and management of AIDS. AIDS Research. 2:141-148, 1986. (Reprint of 39)

51. Vetto, J.T., Papa, M.Z., Lotze, M.T., Chang, A.E., Rosenberg, S.A.: Reduction of toxicity of interleukin-2 and lymphokine activated killer cells in humans by the administration of corticosteroid. J. Clin. Oncol. 5:496-503, 1987.

52. Lotze, M.T., Carrasquillo, J.A., Weinstein J.N., Bryant, G.J., Perentesis, P., Reynolds, J.C., Matis, L.A., Eger, R.R., Keenan, A.M., Hellstrom, I.,Hellstrom, K-E,Larson,S.M.:Monoclonal antibody imaging of human melanoma: Radio-immunodetection by subcutaneous or systemic injection.Ann. Surg. 204:223-35, 1986.

53. Skibber, J.M., Lotze, M.T., Seipp, C.A., Salcido, R., Rosenberg, S.A.: Limb sparing surgery for soft tissue sarcomas: Analysis of wound related morbidity. Surgery 102:447-452, 1987.

54. Roberts, K., Lotze, M.T., Rosenberg, S.A.: The lymphokine activated killer cell. Separation and functional studies of the human precursor and effector cell. Cancer Res. 47:4366-4371, 1987.

55. Lotze, M.T.: The role of lymph node dissection in the treatment of metastatic cancer to regional lymph nodes. (In): Surgical Cure of Metastatic Cancer. Rosenberg, S.A. (ed), J.B. Lippincott Co., Philadelphia, 1987.

56. Skibber, J.M., Lotze, M.T., Muul, L.M., Uppenkamp, I.K., Ross, W., Rosenberg, S.A.: Human lymphokine-activated killer cells: Further isolation and characterization of the precursor and effector cell. Nat. Immunity and Cell Growth Reg. 6:291-305, 1987.

57. Cohen, P.J., Lotze, M.T., Roberts, J.R., Rosenberg, S.A., Jaffee, E.S.: The immunopathology of sequential tumor biopsies in patients treated with interleukin-2. Correlation of response with T cell infiltration and HLA-DR expression. Amer. J. Pathol. 129:208-216, 1987.

58. Lee, R.E., Lotze, M.T., Skibber, J.M., Tucker, E., Bonow, R.O., Ognibene, F.P., Carrasquillo, J.A., Shelhammer, J.H., Parrillo, J.E., Rosenberg, S.A.: Cardiorespiratory effects of immunotherapy with interleukin-2. J. Clin. Oncol., 7:7-20, 1989.

59. Gaspari, A., Lotze, M.T., Rosenberg, S.A., Stern, J.B., Katz, S.I.: Cutaneous changes associated with interleukin-2 administration; Development of erythro-derma and cutaneous lymphocytic infiltration. J. Amer. Med. Association. 258:1624-1629, 1987.

60. Roberts, K., Lotze, M.T.: Interleukin-2 promotion of conjugate formation by purified LAK

precursors and T lymphocytes: Enumeration of cell conjugates by flow cytometry. J. Biol. Resp. Mod. 7:249-266, 1988.

61. Lotze, M.T., Roberts, K., Custer, M.C., Segal, D.A., Rosenberg, S.A.: Specific binding and lysis of human melanoma by IL-2 activated cells coated with anti-T3 and Anti-Fc receptor crosslinked to anti-melanoma antibody: A possible approach to the immunotherapy of human tumors. J. Surg. Res. 42:580-589, 1987.

62. Skibber, J.M., Lotze, M.T., Uppenkamp, I., Ross, W., Rosenberg, S.A.: Identification and expansion of human lymphokine activated killer cells: Implications for the immunotherapy of cancer. J. Surg. Res. 42:613-621, 1987.

63. Lotze, M.T., Custer, M.C., Rosenberg, S.A.: Interleukin 2 (IL-2) administration to humans results in the rapid emigration of a specific lymphocyte subset ($CD2^+$, 3-, 11^+, 16^+) from the peripheral blood. Unpublished.

64. Denicoff, K.D., Rubinow, D.R., Papa, M.Z., Simpson, C., Seipp, C.A., Lotze, M.T., Chang, A.E., Rosenstein, D., Rosenberg, S.A.: The neuropsychiatric effects of IL-2/lymphokine activated killer cell treatment. Ann. Int. Med. 107:293-300, 1987.

65. Rosenberg, S.A., Lotze, M.T., Muul, L.M., Chang, A.E., Avis, F.P., Leitmann, S., Linehan, W.M., Robertson, C.N., Lee, R.E., Rubin, J.T., Seipp, C.A., Simpson, C.G., White, D.E.: Clinical experience with the treatment of 157 patients with advanced cancer using lymphokine activated killer cells and interleukin 2 or high dose interleukin-2 alone. NEJM, 316:889-905, 1987.

66. Lotze, M.T.: Biology of IL-2 and rationale for its clinical application. In Rosenberg, S.A., moderator. New approaches to the immunotherapy of cancer. Ann. Int. Med. 108:853-864, 1988.

67. Lotze, M.T. Treatment of hepatocellular carcinoma. In Dibisceglie, A.M., moderator. Hepatocellular carcinoma. Ann. Int. Med. 108:390-401, 1988.

68. Lotze, M.T., Rosenberg, S.A. Interleukin-2 as a pharmacologic reagent. Lymphokines, Ed.: Kendall Smith, Academic Press; NY, NY. 1988, pp237-294.

69. Rizzoni, W.E., Miller, K., Rick, M., Lotze, M.T. Heparin induced thrombocytopenia and thromboembolism in the perioperative period. Surgery 103: 470-476, 1988.

70. Cotran R., Pober JS, Gimbrone, Jr. M, Springer TA, Wiebke EA, Gaspari AA, Rosenberg SA, Lotze MT. Endothelial activation during interleukin-2 immunotherapy: A possible mechanism for the vascular leak syndrome. J. Immunol. 139:1883-88,1987.

71. Lotze, M.T., Rosenberg, S.A. Interleukin 2 therapy for disseminated cancer (letter to editor). JAMA 257:1729-1731. 1987.

72. Lee, R.E., Gaspari, A.A., Lotze, M.T., Chang, A.E., Rosenberg, S.A. Effects of interleukin-2 on psoriasis. Arch. Derm., 124:1811-1815, 1988.

73. Wiebke, E.A., Lotze, M.T., Rosenberg, S.A. Tumor cell susceptibility to lysis: Marked increase in lysis by tumor infiltrating lymphocytes following target stimulation with interferon-ã and tumor necrosis factor -ã. Implications for immunotherapy. Surgical Forum. 38:436-438, 1987.

74. Weinstein, J.N., Black, D.V., Holton, O.D. III, Covell, D.G., Parker, R.J., Mulshine, J.L., Lotze, M.T., Carrasquillo, J., Eger, R., Lewis, A., Larson, S.A., Keenan, A.M. Delivery of monoclonal antibodies to lymph nodes via the lymphatics. In: The Pharmacology and Toxicology of Proteins, Winkelhade, J.L., Holcenberg, J.S., Eds., NY: Alan R. Liss. 1988.

75. Lotze, M.T., Rosenberg, S.A. The Immunologic Treatment of Cancer. Ca-A Cancer Journal for Clinicians. 38:68-94, 1988.

76. Lotze, M.T., Ross, W.G., Tomita, S., Custer, M.C. Cells invading tumors: Strategies for the identification and expansion of tumor reactive lymphocytes. Transplantation Proceedings. 20:326-331, 1988.

77. Skibber, J.M,., Matter, G.J., Pizzo, P.A., Lotze, M.T. Right lower quadrant pain in young patients with leukemia: A surgical perspective. Ann. Surg, 206:711-716, 1987.

78. Wiebke, E.A., Rosenberg, S.A., Lotze, M.T. Cytokines alter target cell susceptibility to lysis: I. Evaluation of non-MHC restricted effectors reveals differential effects on natural and lymphokine-activated killing. J.Biol.Response Modifiers 9:113-126, 1990.

79. Jablons, D.M., Lotze, M.T. Derivation of cells with lytic activity against fresh and cultured tumors from human bone marrow. J. Surg. Res. 44:404-416, 1988.

80. Wiebke, E.A., Rosenberg, S.A., Lotze, M.T.: Acute immunologic effects of IL-2 in cancer patients: Decreased delayed type hypersensitivity response and decreased proliferative response to soluble antigens. J. Clin. Oncol. 6:1440-9, 1988.

81. Chang AE, Kinsella T, Glatstein E, Baker AR, Sindelar WF, Lotze MT, Danforth Jr. DN, Sugarbaker PH, Lack EE, Steinberg SM, White DE, Rosenberg SA. Adjuvant chemotherapy is effective for patients with high grade extremity sarcomas: Extended follow-up on treated patients. J. Clin. Oncol. 6:1491-1500, 1988.

82. Topalian, S.L., Solomon, D., Avis, F.P., Chang, A.E., Freerksen, D.L., Linehan, W.M., Lotze, M.T., Robertson, C.N., Seipp, C.A., Simon, P., Simpson, C.G., Rosenberg, S.A.: Immunotherapy of patients with advanced cancer using tumor infiltrating lymphocytes and recombinant interleukin-2: A pilot study. J. Clin. Oncol. 6:839-853, 1988.

83. Lotze, M.T.: Surgical management of hepatocellular carcinoma. Gastroenterology Clin. of N.A. 16:613-626, 1987.

84. Lotze, M.T., Tomita, S., Rosenberg, S.A.: Human tumor antigens defined by cytotoxic and proliferative T cells. In: Human Tumor Antigens and Specific Tumor Therapy. Eds. R. Metzgar and M.M Mitchell, 1988.

85. Lotze, M.T.: Book Review: "A history of surgery with emphasis on the Netherlands." By: D. De Moulin. Ann. Int. Med., 109:86-87, 1988.

86. Rosenberg, S.A., Longo, D.L, Lotze, M.T.: Principles of Biologic Therapy. In: Principles and Practices of Oncology. Eds. DeVita, V., Hellman, S., and Rosenberg, S.A. Lippincott, Philadelphia, 1989. pp. 301-348.

87. Del Vecchio, S., Reynolds, J.C., Carrasquillo, J.A., Blasberg, R.G., Neumann, R., Lotze, M.T., Bryant, G.J., Farkas, R.J., Larson, S.M.: Local distribution and concentration of ^{131}I-9.2.27 monoclonal antibody in malignant melanoma following intravenous injection. Cancer Res. 49:2783-2789, 1989.

88. Begley, M.G., Shawker, T.H., Robertson, C.N., Wei, J., Bock, S., Lotze, M.T.: Fournier's gangrene: Diagnosis by scrotal ultrasound examination. Radiology. 169:387-389, 1988.

89. Schwartzentruber, D., Lotze, M.T., Rosenberg, S.A.: Colonic perforation: An unusual complication of therapy with high-dose IL-2. Cancer. 62:2350-2353, 1988.

90. Jablons, D.M., Mule, J.J., McIntosh, J.K., Sehgal, P.B., May, L.T., Huang, C.M., Rosenberg, S.A., Lotze, M.T.: Interleukin-6/Interferon β-2 as a circulating hormone: Induction by cytokine administration in humans. J. Immunol., 142:1542-1547, 1989.

91. Kawakami, Y., Custer, M.C., Rosenberg, S.A., Lotze, M.T.: Interleukin-4 regulates Interleukin-2 induction of lymphokine activated killer activity from human lymphocytes. J. Immunology. 142:3452-3461, 1989.

92. Kawakami, Y., Rosenberg, S.A., Lotze, M.T.: Interleukin-4 promotes the growth of tumor infiltrating lymphocytes specific for human autologous melanoma. J. Exp. Med., 168:2183-2191, 1988.

93. Sakahara, H., Reynolds, J.C., Carrasquillo, J.A., Lora, M.E., Maloney, P.J., Larson, S.M., Neumann, R.D., Lotze, M.T.: In vitro complex formation and biodistribution of mouse antitumor monoclonal antibody in cancer patients. J. Nucl. Med., 30:1311-1317, 1989.

94. Rosenberg, S.A., Packard, B., Aebersold, P.M., Solomon, D., Topalian, S.L., Toy, S., Simon, P., Lotze, M.T., Yang, J.C., Seipp, C.A., Simpson, C., Carter, C., Bock, S., Schwartzentruber, D., Wei, J.P., White, D.E.: Immunotherapy of patients with metastatic melanoma using tumor infiltrating lymphocytes and Interleukin-2: Preliminary report. N. Engl. J. Med., 319:1676-1680. 1988.

95. Rosenberg SA, Lotze MT, Yang JC, Topalian SL, Chang AE, Schwartzentruber DJ, Aebersold P, Leitman S, Linehan WM, Seipp CA, White DE and Steinberg SM. Prospective Randomized Trial of High-Dose Interleukin-2 Alone or In Conjunction with Lymphokine-Activated Killer Cells for the Treatment of Patients with Advanced Cancer. JNCI, 85: 622-632, 1993.

96. Stötter H., Wiebke E.A., Tomita S., Belldegrun A., Topalian S., Rosenberg S.A., Lotze M.T.: Cytokines alter target cell susceptibility to lysis: II. Evaluation of tumor infiltrating lymphocytes. J. Immunol., 142:1676-1773, 1989.

97. Denicoff, K.D., Durkin, T.M., Lotze, M.T., Quinlan, P.E., Davis, C.L., Listwak, S.J., Rosenberg, S.A., Rubinow, D.R.: The neuroendocrine effects of Interleukin-2 treatment. J. Clin. Endocrinology and Metabolism, 69:402-410, 1989.

98. Ognibene, F.P., Rosenberg, S.A., Lotze, M.T., Skibber, J., Parker, M.M., Shelhamer, J.H., and Parrillo, J.E.: Interleukin-2 administration causes reversible hemodynamic changes and left ventricular dysfunction similar to those seen in septic shock. Chest. 94:750-754, 1988.

99. Larson, ST., Carrasquillo, J., Reynolds, J., Deenan, A., Sugarbaker, P.,Colcher, D., Schlom, J., Neumann, R., Hellstrom, I., Hellstrom, K., Mulshine, J.,Lotze, M., and Strudler, P.: The NIH

experience with radiolabeled monoclonal antibodies: lymphoma, melanoma, and colon cancer. In: Radiolabeled monoclonal antibodies for imaging and therapy, SC Srivastava, ed., Plenum Publishing Corp., New York, 1988.

100. Jablons, D.M., McIntosh, J.K., Mule, J.J., Nordan, J.J., Rudikiff, S., and Lotze, M.T.: Induction of Interferon-B_2/ Interleukin-6 (IL-6) by cytokine administration and detection of circulating IL-6 in the tumor-bearing state In: Regulation of the Acute Phase and Immune Responses: A New Cytokine, Ed: Sehgal P.B., Grieninger, G., and Tosato, G., Ann. 557:157-162 Annals New York Acad. Science,1989.

101. McIntosh, J.K., Mule, J.J., Jablons, D.M., Nordan, R.P., Rudikoff, S., Lotze, M.T., Rosenberg, S.A.: The kinetics of IL-6 induction by systemic administration of rhTNF-á in mice. Annals New York Academy of Sciences, 557:572-575, 1989.

102. Lotze, M.T., Kawakami, Y., and Rosenberg, S.A.: Immunotherapy Protocols at the National Cancer Institute: Current Status and Future Prospects. Colloque Inserm 179:153-162, 1989.

103. Laue, L., Lotze, M.T., Chrousos, G.P., Barnes, K., Loriaux, L., and Fleisher,T.A.: Effect of chronic treatment with the glucocorticoid antagonist RU486 in man: toxicity, immunological and hormonal aspects. J. Clin. Endocrinol. 71:1474-80, 1990

104. Kasid, AT., Director, E., Stovroff, M.C., Lotze, M.T., Rosenberg, S.A.: Cytokine regulation of tumor necrosis factor-á and â (lymphotoxin)- messenger RNA expression in human peripheral blood lymphocytes. Cancer Res. 50: 5072-6, 1990.

105. Fisher, B., Keenan, A., Garra, B.S., Steinberg, S.M., White, D.E., DeBisceglie, A.M., Hoofnagle, J.H., Yolles, P., Rosenberg, S.A., Lotze, M.T.: Interleukin-2 induces profound reversible cholestasis: A detailed analysis in treated patients. J. Clin. Oncol. 7:1852-1862, 1990.

106. Rosenberg, S.A., Lotze, M.T., Yang, J.C., Linehan, W.M., Seipp, C., Calabro, S., Karp, S.E., Sherry, R.M., Steinberg, White, D.E.: Combination therapy with Interleukin-2 and Alpha-Interferon for the treatment of patients with advanced cancer. J. Clin. Oncology 7:1863-1874, 1989.

107. Ward, B., McGarvey, C., Lotze, M.T.: Functional outcome following partial or total scapulectomy/Analysis at prolonged follow-up. Arch Surg. 124:537-542, 1989.

108. Bock, S.N., Lee, R.E., Fisher, B., Rubin, J.T., Schwartzentruber, D., Wei, J.P., Callender, D., Yang, J.C., Lotze, M.T., Pizzo, P.A., Rosenberg, S.A.: A prospective randomized trial evaluating prophylatic antibiotics to prevent catheter-related sepsis in patients treated with immunotherapy. J Clin Oncol 8:161-169, 1990.

109. Wade, TP, Lotze, MT: Lymph stasis after lymph node dissection.In: Lymph Stasis: Pathophysiology, Diagnosis, and Treatment, WL Olszewski Ed. Year Book Pub 1991.

110. Custer, M.C., Lotze, M.T.: A biologic assay specific for Interleukin-4: Rapid Fluorescence Assay for IL-4 detection in supernatants and serum, J. Imm. Methods, 128: 109-17, 1990.

111. Lotze, M.T.: Letter to the editor; High output cardiac failure in patients with multiple myeloma. New Eng. J. of Medicine, 320:1419, 1989.

112. Rubin, J.T., Elwood, L., Rosenberg, S.A., Lotze, M.T.: Immunohistochemical Correlates of Response to Recombinant Interleukin-2 Based Immunotherapy, Cancer Research, 49:7086-7092, 1989.

113. Jablons, D., Bolton, E., Mertins, S., Rubin, M., Pizzo, P., Rosenberg, S.A., Lotze, M.T.:Interleukin-2 Based Immunotherapy Alters Circulating Neutrophil Fc Receptor Expression and Chemotaxis. J. Immunology, 144:3630-3636, 1990.

114. Culver, K., Cornetta, K., Morecki, S., Aebersold, P., Freeman, S., Kasid, A., Morgan, R., Lotze, M.T., Rosenberg, S.A., Anderson, W.F., Blaese, R.M.: Gene Therapy: Lymphocytes As Cellular Vehicles For Gene Transfer In Mouse And Man. Proc.Nat.Acad.Sci. 88:3155-3159, 1992.

115. Kragel, A.H., Travis, W.D., Feinberg, L., Pittalugia, S., Striker, L.M., Roberts, W.C., Lotze, M.T., Yang, J.J., Rosenberg, S.A.: Pathologic Findings Associated with Interleukin-2 Based Immunotherapy For Cancer: A Postmortem Study of 19 Patients. Human Pathology, 21:493-502, 1990.

116. Rosenberg, S.A., Lotze, M.T., Yang, J.C., Aebersold, P.M., Linehan, W.M., Seipp, C.A., White, D.E.: Experience With The Use Of High Dose Interleukin-2 In The Treatment Of 652 Patients With Cancer. Ann. Surg, 210:474-485, 1989.

117. Huang, C.M., Ruddle, M., Sliva, C., Elin, R.J., Lotze, M.T., Rosenberg, S.A.: Changes In Laboratory Results With Interleukin-2 Therapy Administered To Cancer Patients. Clin. Chemistry, 36:431-434, 1990.

118. Lotze, M.T.: Interleukin-2 Based Immunotherapy Of Malignant Melanoma, in Therapy of Advanced Melanoma Ed. P. Rumke, S. Karger AG, Basel, Pigment Cell 10: 163-182, 1990.

119. McIntosh, J.E., Jablons, D.M., Mule, J.J., Nordan, R. P., Rudikoff, S., Lotze, M.T., Rosenberg, S.A.: In Vivo Induction of IL-6 by Administration of Exogenous Cytokins and Detection of De Novo Serum Levels of IL-6 in Tumor-Bearing Mice. J. Immunology., 143:162-167, 1989.

120. Kasid, A., Morecki, S., Aebersold, P., Cornetta, K., Culver, K., Freeman, S., Director, E., Lotze, M.T., Blaese, R.M., Anderson, F., Rosenberg, S.A.: Human Gene Transfer: Characterization of human tumor infiltrating lymphocytes as vehicles for retroviral mediated gene transfer in man. Pro. Nat. Acad. Sci., 87:473-477, 1990.

121. Rubin, J.T., Rosenberg, S.A., Lotze, M.T.: The efficacy of high dose IL-2 based immunotherapy in man. in Rees RC Ed IRL Press, pp. 139-159, 1990. The Biology and Clinical Applications of Interleukin-2.

122. Stotter, H., Lotze, M.T.: Cytolytic effector cells against human tumors: Distinguishing phenotype and function. Cancer Cells, 2:44-56, 1990.

123. Lotze, M.T., Custer, M.C., Bolton, E.S., Wiebke, E.A., Kawakami, Y., Rosenberg, S.A.: Mechanisms of immunologic antitumor therapy: lessons from the laboratory and clinical applications. Human Immunology, 28:198-207, 1990.

124. Lotze, M.T., Jablons, D.M., Rubin, J.T., Chang, A.E., Rosenberg, S.A.: Cytokine therapy of patients with cancer. Progress In Immunology, 7:1213-1220, 1990.

125. Jablons DM, Donohue R, Kawakami Y, Young JD, Lotze MT. Interleukin-3 induces proliferation but not lymphokine activated killer activity from human and murine mononuclear cells. Eur Cytokine Netw. 1990 May-Jun;1(2):91-8.

126. Kawakami, Y., Haas, G.P., Lotze, M.T.: Expansion of Tumor Infiltrating Lymphocytes from Human Tumors Using the T cell Growth Factors Interleukin-2 and Interleukin-4. J. Immunotherapy 14:336-47, 1993.

126a. Lotze, M.T.: Biological Response Modifiers in Cancer Therapy (book review). Immunology Today, 11:143, 1990.

127. Lotze, M.T.: Interleukin-2-Basic Principles, in Principles and Practice of Biologic Therapy, DeVita, V., Hellman, S., Rosenberg, S.A. J Lippincott, Phila., 1991.

128. Lotze, M.T.: Cancer Treatment with Interleukin-2, Principles and Practice of Biologic Therapy, ed DeVita, V, Hellman, S., Rosenberg, S.A. J Lippincott, Philadelphia, 1991.

129. Lotze, M.T.: Disturbing Homeostasis: Recent results of ongoing immunotherapy trials at the NCI. Biotechnology Therapeutics, 1:125-162, 1989.

130. Lotze, M.T.: Book Review, Biologic Response Modifiers in Cancer Therapy. Immunology Today. 11:143, 1990.

131. Lotze, M.T.: Preface - Current paradigms in cellular immunology: Implications for immunity to cancer. In Cellular Immunology and the Immunotherapy of Cancer. ed Lotze, M.T. and Finn, O., Wiley-Liss, New York, 1990.

132. Lotze, M.T., Custer, M.C., Kawakami, Y., Stotter, H.,Rubin, J.T., Bolton, E.S., Guedez, L. and Sanda, M.G.: T cell growth factors and the expansion of lymphoid cells with antitumor activity in vitro and in vivo. In Cellular Immunity and the Immunotherapy of Cancer. eds. Lotze, M.T.

and Finn, O.J. Wiley-Liss, New York, 1990.

133. Stötter, H., Custer, M.C., Bolton, E.S., Guedez, L. and Lotze, M.T.: Interleukin-7 induces human lymphokine activated killer (LAK) cells and is regulated by interleukin-4. J. Immunol 146: 150-144, 1991.

134. Lotze, M.T. and Finn, O.J.: Current Paradigms In Cellular Immunity: Implications for immunity to cancer. Immunology Today. 11:190 - 3, 1990

135. Lotze, M.T. and Rosenberg S. A.: The Use of Lymphokines in therapy, in Progress in Allergy and Clinical Immunology ed. W.J. Pickles, B. M. Stadler, C. Dahinden et al; Hogrefe & Huber, Toronto pp. 529-536, 1989.

136. Rosenberg, S.A., Aebersold, P., Cornetta, K., Kasid, A., Morgan, R.A., Moen, R., Karson, E.M., Lotze, M.T., Yang, J.C., Topalian, S.L., Merino, M.J., Culver, K., Miller, A.D., Blaese, R.M. and Anderson, W.: Gene transfer into humans: Immunotherapy of patients with advanced melanoma using tumor infiltrating lymphocytes modified by retroviral gene transduction. N. Engl. J. Med. 323: 570-578, 1990.

137. Stötter, H. and Lotze, M.T.: Human Lymphokine Activated Killer Activity: Role of Interleukin-2, Interleukin-4, and Interleukin-7. Arch Surg 126:1525-30, 1991.

138. Aebersold, P., Hyatt, S., Johnson, S., Hinesk, Korcak, L., Sanders, M., Lotze, M., Topalian, S., Yang, J. and Rosenberg, SA: Lysis of autologous melanoma cells by tumor infiltrating lymphocytes: Association with clinical response. Journal of the National Cancer Institute, 83: 932-937, 1991.

139. Feuerstein, I. M., Chow, C. K., Miller, D.L., Carrasquillo.J.A., Lotze, M.T., Radiologic Findings in Hepatocellular Carcinoma: The National Institutes of Health Experience in Hepatocellular Carcinoma, Ed. Tabor E, DiBesceglie A and Purcell RH. pp. 255-272, 1991.

140. Spencer W. F., Linehan, W. M., Walther, M.M., Lotze, M.T., Topalian, S., Yang, J., Merino, M. J., Lange, J. R., Pockaj, B.A., and Rosenberg, S.A.: The Role of Pre-nephrectomy IL-2 based immunotherapy in patients with metastatic renal cell carcinoma, J. Urology.147:24-30, 1992.

141. Wong, H. L., Lotze, M.T., Wahl, L.M., Wahl, S.M.: Administration of recombinant IL-4 to humans regulates gene expression, phenotype, and function in circulating monocytes. J. Immunol. 148:2118-2125, 1992.

142. Lotze, M.T.: T cell growth factors and the treatment of patients with cancer. Clinical Immunology and Immunopathology 62:s47-s54, 1992.

143. Edington, H.D., and Lotze, M.T.: Immunotherapy of human sarcomas. Postgraduate General Surgery 3:36-41, 1991.

144. Lotze, M.T.: Interleukin-2 in Human Cytokines, ed Aggarwal BB, Gutterman JV, Blackwell Scientific Publications, Cambridge, 1991.

145. Rubin J. T. and Lotze M.T.: Acute Gastric Mucosal Injury Associated with Systemic Administration of Interleukin-4. Surgery 111:274-280, 1991.

146. Lotze, M.T.: Concerning repeat hepatic resections for colorectal cancer (letter to editor) Surgery 109:347-348, 1991.

147. Lotze, MT. Healing Arts (journal review article-Biotherapy and Molecular Biotherapy) Nature 347: 587-588, 1990.

148. Lotze MT and Kramarski CM. Shoulder immobilizer for axillary nodal dissection: A "safe" device. Contemporary Oncology, 1991.

149. Lotze MT. Sui Generis (journal review article). Nature 1991.

150. Lotze MT. Role of IL-4 in the antitumor response in H. Spits,ed. Interleukin-4 Raven Press, 1992, Chapter 15, 237-262.

151. Choyke PL, Miller DL, Lotze MT, Whiteis J, Ebbat B, and Rosenberg SA. Delayed reaction to intravenous contrast media following Interleukin-2 immunotherapy. Radiology 183:111-114, 1992.

152. Lotze MT. Trials and Tribulations: The Cost of Developing Gene Therapies (Editorial) FASEB Jl 5:3013-3014, 1991.

153. Emery BE, Igarashi Y, White MV, Mullol J, Berkebile C, Peden D, Lotze MT, and Kaliner MA. The effect of IL-4 Upon Human Nasal Mucosal Responses. Jl Allergy and Clinical Immunology, 90:772-81, 1992.

154. Lotze MT. Clinical Protocol: The treatment of patients with melanoma using IL-2, IL-4, and tumor infiltrating lymphocytes. Human Gene Therapy, 3: 167-177, 1992.

155. Lotze MT, Zeh HJ, Elder EM, Cai Q, Pippin BA, Whiteside TL and Herberman RB. Use of T-cell Growth Factors (IL-2 to IL-12) in the investigation and treatment of melanoma. J. Immunotherapy, 12: 212-217, 1992

156. Weidmann E, Elder EM, Trucco M, Lotze MT, and Whiteside TL. Usage of the T-Cell receptor Vâ chain genes in fresh and cultured tumor-infiltrating lymphocytes from human melanoma. Int. J. Cancer 54:383-390, 1993.

157. Tahara H, Mueller GM, Ricordi C, Robbins PD, Lotze MT. Islet cell transplantation facilitated

by gene transfer. Transplant. Proc. 24:2975-2976, 1992.

158. Wong HL,Costa GL, Lotze MT,Wahl SM.IL-4 differentially regulates monocyte IL-1 family gene expression and synthesis in vitro and in vivo. J Exp Med177:775-81,1993.

159. Richards JM and Lotze MT. Interleukin-2 Therapy:Current Status and Future Directions. Contemporary Oncology 2:34-44, 1992.

160. Lotze MT. Keystone Symposium. Melanoma and Biology of the Neural Crest. Melanoma Res, 2:131-138, 1992.

161. Lange JR, Raubitschek AA, Pockaj BA, Spencer WF, Lotze MT, Topalian SL, Yang JC and Rosenberg SA. A pilot study of combination of Interleukin-2 based immunotherapy and radiation therapy. J. Immunotherapy, 12: 265-271, 1992.

162. Sanda MG, Yang JC, Topalian SL, Groves ES, Childs A, Rubens R, deSmet MD, Schwartzentruber DJ, White DE, Lotze MT and Rosenberg SA. Intravenous administration of recombinant human macrophage colony-stimulating factor to patients with metastatic cancer: A Phase I Study. J Clin Onc, 10: 1643-1649, 1992.

163. Rubin JT, Duquesnoy R, Day R, Simonis TB, Rosenberg SA and Lotze MT. HLA-DQ1 is associated with clinical response and prolonged survival of patients with melanoma who are treated with interleukin-2 based immunotherapy in humans. Therapeutic Immunology, 2:1-6, 1995.

164. Lotze MT, Carr B and Flickenger J. Hepatobiliary Neoplasms in Principals and Practices of Oncology, Rosenberg, DeVita and Hellman (ed) J. B. Lippincott, 883-914, 1993.

165. Miller DL and Lotze MT. A Plea for a Standard Standard. Intraarterial chemotherapy for hepatic neoplasms. Radiology 188:19-20, 1993.

166. Itoh I, Storkus WJ, Gorelik E and Lotze MT. Partial purification of murine tumor-associated peptide epitopes common to histologically distict tumors, melanoma and sarcoma that are presented by H-2K^b and recognized by CD8+ Tumor Infiltrating Lymphocytes. J Immunology 153:1202-15, 1994.

167. Lotze MT, Storkus WJ, Hurd SD, Maeurer MJ, Kirkwood JM. Immunotherapy of melanoma. Melanoma Research 3:5-6, 1993.

168. Edington H and Lotze MT. Immunotherapy, in Colorectal Cancer, ed. HJ Wanebo, Mosby, St. Louis, pp540-564, 1993.

169. Edington HD and Lotze MT. Cellular Immunotherapy for Colorectal Cancer.in Cohen AM, Winawer SJ, Friedman MA, Gunderson LL (editors), Cancer of the Colon, Rectum and Anus;

McGraw-Hill, Inc., NY, pp949-959, 1995.

170. Robbins PD, Tahara H, Mueller G, Hung G, Bahnson A, Zitvogel L, Galea-Lauri J, Ohashi T, Patrene K, Boggs SS, Evans CH, Barranger JA and Lotze MT. Retroviral Vectors for use in Human Gene Therapy for Cancer, Gaucher Disease, and Arthritis. Annals of the New York Acad. Sci.716:72-88, 1994.

171. Zeh HJ, Hurd S, Storkus WJ, and Lotze MT. Interleukin 12 Promotes the Proliferation and Cytolytic Maturation of Immune Effectors: Implications for the Immunotherapy of Cancer. J Immunotherapy 14:155-161, 1993.

172. Zeh HJ, Tahara H, and Lotze MT. Interleukin-12. Chapter 13 in The Cytokine Handbook, Second Edition; pp 239-256; Editor, A. Thomson, Academic Press, Ltd; London; 1994.

173. Nastala CL, Edington HD, Storkus WJ and Lotze MT. Recombinant Interleukin-12 (r-mIL-12) Mediates Regression of Both Subcutaneous and Metastatic Murine Tumors. Surgical Forum 44:518-521, 1993.

174. Nastala CL, Edington H, Storkus W, McKinney TG, Brunda M, Gately M, Wolf SF, chrieber R and Lotze MT. Recombinant Interleukin-12 Induces Tumor Regression in Murine Models: Interferon-Gamma But Not Nitric Oxide Dependent Effects. J. Immunol 153,:1697-1706, 1994.

175. Tahara H, Zeh H, Storkus WJ, Pappo I, Watkins S, Gately MK, Gubler U, Wolf SF, Robbins PD and Lotze MT. Fibroblasts Genetically Engineered to Secrete Interleukin-12 can Suppress Tumor Growth In Vivo and Induce Antitumor Immunity To A Murine Melanoma. Cancer Res. 54:182-189, 1994.

176. Lotze MT. Transplantation and Adoptive Cellular Therapy of Cancer: The Role of T cell Growth Factors. Cell Transplantation, 2:33-47, 1993.

177. Storkus W. J., Zeh III, H. J., Salter R. D., Lotze, M. T. Identification of T cell epitopes: Rapid isolation of class I-presented peptides from viable cells by mild acid elution. J. Immunother.14:94-103, 1993.

178. Storkus W. J., Zeh III, H. J., Salter R. D., Lotze, M. T. Isolation of human melanoma peptides recognized by class I restricted tumor infiltrating T lymphocytes. J. Immunol.151:3719-3727, 1993.

179. Lotze MT, Rubin JT and Zeh HJ. New Biologic Agents Come to Bat for Cancer Therapy. Current Science, 4: 1116-1123, 1992.

180. Marincola FM, Venzon D, White D, Rubin JT, Lotze MT, Simonis TB, Balkisson J, Rosenberg SA, Parkinson DR. HLA association with response and toxicity in melanoma patients treated with interleukin-2 based immunotherapy. Cancer Research 52:6561-6, 1992.

181. Rubin JT, Lotze MT. Immune function and dysfunction. A primer for the Radiologist. Radiologic Clinics of North America 30:507-23, 1992.

182. White MV, Igarashi Y, Emery BE, Lotze MT, Kaliner MA. Effects of *in vivo* administration of interleukin-2 (IL-2) and IL-4 alone and in combination, on *ex vivo* human basophil histamine release. Blood 79:1491-5, 1992.

183. Oppenheim MH, Lotze MT. Interleukin-2: Solid Tumor Therapy. Oncology 51:154-69, 1994.

184. Leder GH, Oppenheim M, Rosenstein M, Shah N, Hoffman R, Lotze MT, Beger HG. Inhibition of nitric oxide synthesis does not improve Interleukin-2-mediated antitumor effects in vivo. Eur Surg Res 28:167-78, 1996.

185. Lotze MT, Finn OJ. Cellular immunity and the immunotherapy of cancer. J.Immunother. 14:79-87, 1993.

186. Lotze MT, Agarwala SS, Kirkwood JM.Clinical Development of IL-2. Oncology 1993.

187. Zeh HJ, Salter RD, Techtor M, Leder G, Stuber G, Modrow S, Lotze MT, Storkus WJ. Flow cytometric determination of peptide-Class I complex formation: Identification of p53 peptides binding HLA-A2. Human Immunology 39:79-86, 1994.

188. Hunt JD, Pippin AB, Landreneau RJ, Jacob WF, Lotze MT, Siegfried JM. Transfer and expression of the human Interleukin-4 gene in carcinoma and stromal cell strains derived from lung cancer patients. J Immunotherapy 14:314-21, 1993.

189. Irshaid Y, Adedoyin A, Lotze M, Branch R. Monoacetyldapsone inhibition of dapsone hydroxylation by human and rat liver microsomes. Drug Metab.Disp. 22:161-4, 1994.

190. Leder GH, Storkus WJ, Zeh HJ, Lotze MT. Can p53 peptides serve as tumor-specific antigens? Peptides 1992, Ed. SH Schneider, AN Eberle. ESCIM Science Pub. 1993.

191. Rosenberg SA, Lotze MT, Yang JC, Topalian SL, Chang AE, Schwartzentruber DJ, Aebersold P, Leitman S, Linehan WM, Seipp CA et al.Prospective randomized trial of high-dose IL-2 alone or in conjunction with lymphokine-activated killer cells for the treatment of patients with advanced cancer. Jl. Nat. Cancer Inst. 85:622-32, 1993.

192. Edington HD, Lotze MT. Interleukin 7 in Cytokine Handbook, ed. Angus Thompson, 1994.

193. Lotze MT. Biologic Response Modifiers and the Treatment of Infectious Diseases in Surgical Infectious Diseases 3rd Edition; ed. RJ Howard, RL Simmons, 1994; Appleton and Lange, Norwalk, CN; pp 595-616.

194. Lotze MT. Molecular biology and clinical applications to cancer. Curr. Opinion in General Surgery 2:245-254, 1994.

195. Lotze MT. What's New in Surgical Oncology. Bull. Amer. Coll. Surg.79:42-47, 1994.

196. Pappo I, Tahara H, Nishihara K, Robbins PD, Gately MK, Wolf SF, Lotze MT. Administration of systemic or local interleukin-2 enhances the antitumor effects of interleukin-12 gene therapy. J. Surg. Res, 57:218-226, 1995.

197. Lotze MT. Soft Tissue Sarcomas: Diagnosis and Treatment Ed. John H. Raaf (Book Review). N. Engl. J. Med. 329:1284-1285, 1993.

198. Qin L, Chavin KD, Tahara H, Robbins PD, Lotze MT, Favarro J, Woodward J, Ding Y, Bromberg JS. Retrovirus-Mediated Transfer of Viral Interleukin-10 Gene Prolongs Murine Cardiac Allograft Survival. J Immunology 156:2316-2324, 1996.

199. Leder GH, Oppenheim M, Rosenstein M, Shah N, Hoffman R, Simmons R, Lotze MT. The IL-2 induced nitric oxide production but not the IL-2 induced capillary leak syndrome is decreased by aminoguanidine.3rd International Nitric Oxide Mtg.

200. Stuber G, Leder GH, Storkus WJ, Lotze MT, Modrow S, Szekely L, Wolf H, Klein E, Karre K, and Klein G. Identification of wild-type and mutant p53 peptides binding to HLA-A2 assessed by a peptide loading deficient cell line assay and a novel MHC Class I peptide binding assay. Eur. J. Immunol. 24:765-8, 1994.

201. Maeurer MJ, Hurd S, Martin D, Storkus WJ,, Lotze MT. Cytolytic T-cell clones define HLA-A2 restricted human cutaneous melanoma peptide epitopes - correlation with T-cell receptor usage. The Cancer Journal 2:162-170, 1995.

202. Maeurer MJ, Storkus WJ, Lotze MT. Tumor Vaccines. Principles of Clinical Immunology, Robert Rich, Editor, 1995.

203. Shaw S, Luce GG, Gilks WR, Anderson K, Ault K, Bochner BS, Boumsell L, Denning SM, Engleman EG, Fleisher T, Freedman AS, Fox DA, Gailit J, Gutierrez-Ramos JG, Hurtubise PE, Lansdorp P, Lotze MT, Mawhorter S, Marti G, Matsuo Y, Minowda J, Michelson A, Picker L, Ritz J, Roos E, van der Schoot CE, Springer TA, Tedder TF, Telen MJ, Thompson JX, Valent P. Leukocyte differentiation antigen database. in Leukocyte Typing V; edited by Schlossman S, Boumsell L, Gilks W, Harlan J, Kishimoto T, Morimoto C, Ritz J, Shaw S, Silverstein R, Springer T, Tedder T, and Todd R. Oxford University Press, 1994.

204. Pockaj BA, Yang JC, Lotze MT, Lange JR, Spencer WF, Steinberg SM, Topalian SL, Schwartzentruber DJ, White DE, Rosenberg SA. A prospective randomized trial evaluating colloid versus crystalloid resuscitation in the treatment of the vascular leak syndrome associated with Interleukin-2 therapy. J. Immunotherapy 15:22-28, 1994.

205. Zitvogel L, Tahara H, Robbins PD, Storkus WJ, Wolf SF, Gately M, Lotze MT.Construction and characterization of retroviral vectors expressing biologically active human Interleukin 12 (IL-12). Human Gene Therapy 5:1493-1506, 1994.

206. Tahara H, Lotze MT. Antitumor effect of interleukin-12 (IL-12): Applications for the immunotherapy and gene therapy of cancer. Gene Therapy 2:96-106, 1995.

207. Thomson AW, Lotze MT. Interleukins 13-15. Chapter 14; pp 257-264 in The Cytokine Handbook, Second Edition; Editor, A. Thomson, Academic Press, Ltd; London; 1994.

208. Pippin BA, Rosenstein M, Jacob WF, Chiang Y, Lotze MT. Local IL-4 delivery enhances immune reactivity to murine tumors: gene therapy in combination with IL-2. Cancer Gene Therapy, 1:1-7, 1994.

209. Lotze MT, Rubin JT. Gene Therapy of Cancer: A pilot study of IL-4-gene-modified fibroblasts admixed with autologous tumor to elicit an immune response. Human Gene Therapy 5:41-56, 1994.

210. Strumph PS, Carty SE, Rilo HLR, Carroll PB, Ricordi C, Lotze MT. Interleukin-1 receptor antagonist is not detrimental to human islet cell insulin secretion but decreases insulin content. Transplantation Proceedings 26: 694, 1994.

211. Qin L, Chavin KD, Ding Y, Favaro JP, Woodward JE, Lin J, Tahara H, Robbins P, Shaked A, Ho D, Sapolsky R, Lotze MT, Bromberg JS. Multiple vectors effectively achieve gene transfer in a murine cardiac transplantation model. Transplantation 59:809-816, 1995.

212. Nishihara K, Barth RF, Wilkie N, Lang JC, Oda Y, Kikuchi H, Everson MP, Lotze MT. Increased in vitro and in vivo tumoricidal activity of a genetically engineered macrophage cell

line by an exogenous IFN-ã, IL-4, IL-6 or TNF-á gene expression. Cancer Gene Therapy, 2:113-124, 1995.

213. Storkus WJ, Lotze MT. Biology of Tumor Antigens. B. Tumor Antigens Recognized by Immune Cells In Biologic Therapy of Cancer, ed. DeVita V, Hellman S, Rosenberg SA. JB Lippincott, Phila. 1995.

214. Nastala CL, McKinney BA, Edington HD, Tahara H, Gately MK, Storkus WJ, Lotze MT. Interleukin-12 (IL-12) induces specific anti-tumor immunity in animals bearing established subcutaneous murine sarcoma. Surgical Forum, 80:492-494, 1994.

215. Höhnke C, Nastala CL, Tahara H, Suzuki T, Edington HD, Lotze MT. The use of gene transfer of viral IL-10 to facilitate allogeneic skin transplantation. Surgical Forum, 80:776-779, 1994.

216. Castelli C, Storkus WJ, Maeurer MJ, Martin DM, Huang EC, Pramanik BN, Nagabushan TL, Parmiani G, Lotze MT. Mass spectrometric identification of a naturally-processed melanoma peptide recognized by CD8+ cytotoxic T lymphocytes. J Exp Med,181:363-368, 1995.

217. Lotze MT. Interleukin 2 - Preclinical Studies. In Biologic therapy of Cancer, Ed. Devita V., Hellman S, Rosenberg SA. JB Lippincott, Phila. 1995.

218. Maeurer MJ, Martin DM, Castelli C, Elder E, Leder E, Storkus WJ, Lotze MT. Host immune response in renal cell cancer. IL-4 and IL-10 mRNA are frequently detected in freshly collected tumor infiltrating lymphocytes. Cancer Immunology and Immunotherapy 41:11-121, 1995.

219. Maeurer MJ, Storkus WJ, Lotze MT. New avenues of cancer treatment: Modulation of host anti-tumor directed immune responses by IL-4 and IL-12 in renal cell cancer. In Biology of Renal Cell Carcinoma, Ed. RM Bukowski, JH Finke, Klein EA.Springer-Verlag, New York, pp161-174, 1995.

220. Cai Q, Rubin JT, Lotze MT. Genetically marking human cells - Results of the first clinical gene transfer studies. Cancer Gene Therapy, 2:125-136, 1995.

221. Tahara H, Zitvogel L, Storkus WJ, Zeh HJ, McKinney TG, Schreiber RD, Gubler U, Gately MK, Robbins PD, Lotze MT. Effective eradication of established murine tumors with therapy using a polycistronic retroviral vector. J. Immunol. 154:6466-6474, 1995.

222. Chavin KD, Qin L, Tahara H, Robbins PD, Lotze MT, Bromberg JS. Gene transfer of TGF-â and viral IL-10 prolong cardiac allograft survival. Surg. Forum 44:404-9,1993.

223. Frassanito MA, Mayordomo JI, DeLeo RM, Storkus WJ, Lotze MT, DeLeo AB. Identification of Meth A sarcoma-derived class I MHC-associated peptides recognized by a specific CD8+ CTL. Cancer Research 55:124-128, 1995.

224. Lotze MT, Rubin JT, Whiteside TL, Herberman RB. Cytokine and Cellular Mediated

Immunotherapy of Cancer. Chapter 122 in Principles of Clinical Immunology, pp1919-1930; Robert Rich, Editor, Mosby Yearbook, Inc., St. Louis, 1996.

225. Tahara H, Lotze MT. Gene Therapy for Adult Cancers: Advances in Immunologic Approaches Using Cytokines. Chapter 15 in Somatic Gene Therapy, pp 263-286; Patricia L. Chang, Editor, CRC Press, Inc. Boca Raton, FL, 1994.

226. Suzuki T, Tahara H, Robbins P, Narula S, Moore K, Lotze MT. The human herpes virus 4 cIL-10 homologue, vIL-10 induces local anergy to tumor allo and autografts. J. Exp. Med.182:477-486, 1995.

227. Zitvogel L, Tahara H, Robbins PD, Storkus WJ, Clark M, Nalesnik M, Gately M, Lotze MT. Cancer immunotherapy of established tumors with Interleukin-12 (IL-12): Effective delivery by genetically engineered fibroblasts. J. Immunology 155:1393-1403, 1995.

228. Di Bisceglie AM, Simpson LH, Lotze MT, Hoofnagle JH. Development of hepatocellular carcinoma among patients with chronic liver disease due to Hepatitis C viral infection. J Clin Gastroenterol 19:222-226, 1994.

229. Schwarz RE, Reynolds JC, Lotze MT. "Pseudo-pseudotumor" of the liver. Adenocarcinoma presenting as an inflammatory pseudotumor. Digestive Diseases & Sciences 39:2679-84, 1994.

230. Goydos JS, Lotze MT. Biologic Therapy: Current and Future Considerations. Chapter 73 in Surgery for Gastrointestinal Cancer: A Multidisciplinary Approach. pp831-848, Harold J. Wanebo, editor; Lippincott-Raven Publishers; Philadelphia; 1997.

231. Suminami Y, Elder EM, Lotze MT, Whiteside TL. *In situ* IL-4 gene expression in cancer patients treated with a genetically-modified tumor vaccine. Journal of Immunotherapy 17:238-248, 1995.

232. Elder EM, Lotze MT, Whiteside TL. Successful culture of cytokine gene-modified human dermal fibroblasts for biologic therapy of patients with cancer. Human Gene Therapy 7:479-487, 1996.

233. Mayordomo JI, Zorina T, Storkus WJ, Celluzzi C, Falo LD, Melief CJ, Ildstad ST. Kast WM, DeLeo A, Lotze MT. Bone marrow-derived dendritic cells pulsed with tumor peptides effectively treat established murine tumors. Nature - Medicine 1(12):1297-1302, 1995

234. Myers JN, Yasumura S, Suminami Y, Hirabayashi H, Lin W-C, Johnson JT, Lotze MT and Whiteside TL. Growth stimulation of human head and neck squamous cell carcinoma cell lines by Interleukin-4.Clin. Cancer Research 2:127-136, 1996.

235. Maeurer MJ, Wolter W, Zitvogel L, Storkus WJ, Lotze MT. The role of Interleukin-7 in the expansion of colon cancer - specific and MHC Class II restricted tumor infiltrating lymphocytes (TIL) in vitro and in vivo. Cancer Gene Therapy, 1995

236. Maeurer MJ, Storkus WJ, Kirkwood JM, Lotze MT. Immunization with melanoma derived peptide T-cell epitopes represents a new treatment option for patients with melanoma. Melanoma Research,6:11-24, 1996.

237. Maeurer MJ, Martin D, Elder E, Storkus WJ, Lotze MT. Detection of naturally processed and HLA-A1 presented melanoma T-cell epitopes defined by CD8+ T-cells releasing GM-CSF, but not mediating target cytolysis. Clinical Cancer Research 2:87-96, 1996.

238. Maeurer MJ, Gollin S, Storkus WJ, Swaney W, Martin D, Castelli C, Salter R, Knuth A, Lotze MT. Tumor escape from immune recognition.Loss of HLA-A2 melanoma cell surface expression is associated with a complex rearrangement of the short arm of chromosome 6. Clin. Cancer Res. 2:641-652, 1996.

239. Celluzzi CM, Mayordomo JI, Storkus WJ, Lotze MT, Falo LD. Peptide-pulsed dendritic cells induce antigen specific CTL-mediated protective tumor immunity. J Exp Med 183:283-287, 1996.

240. Zitvogel L, Mayordomo JI, Tjandrawan T, DeLeo AB, Clarke ML, Lotze MT, Storkus WJ. Therapy of Murine Tumors with Tumor Peptide-pulsed Dendritic Cells: Dependence on T Cells, B7 Costimulation, and T Helper Cell 1-associated Cytokines. J Exp Med 183:87-97, 1996.

241. Rubin JT, Lotze MT, Rosenfelder D, Brumfield A, Howells R, Schwartz R, Sylvestri S, Sammon J, Bron K, Orens P, Zajko A, Swanson D, Day R. Treatment of hepatic-metastatic colorectal cancer with a chemotherapeutic emulsion: interim results of a phase I trial. Ann Surg Oncol 2:351-359, 1995.

242. Zitvogel L, Lotze MT. Role of Interleukin-12 (IL-12) as an antitumor agent: Experimental biology and clinical application. Research Immunology 146:628-637, 1995.

243. Pippin BA, Lotze MT. Cytokine Gene Therapy of Cancer in Aggarwal BB, Puri RK; Human Cytokines: Their Role in Disease and Therapy; Blackwell Science, Cambridge, pp675-688; 1995.

244. Lotze MT. Interleukin-2. In *Growth Factors and Cytokines in Health and Disease.* Ed. Derek LeRoith and Carolyn Bondy, JAI Press, Inc; Greenwich, CT; 199; pp1-47.

245. Tahara H, Lotze MT, Robbins PD, Storkus WJ, Zitvogel L. Clinical Protocol: IL-12 gene therapy using direct injection of tumors with genetically engineered autologous fibroblasts. Human Gene Therapy 6:1607-1624, 1995.

246. Leder GH, Oppenheim M, Rosenstein M, Lotze MT, Beger HG. Addition of IL-12 to low dose Interleukin 2 treatment improves antitumor efficacy in vivo. Z. Gastoenter 33:499-502, 1995.

247. Lotze MT. A new perspective: Cytokine Gene Therapy of Cancer. Cancer J from Scientific American 2:63-72, 1996.

248. Lotze MT, Kirkwood JM. Melanoma and other tumors of the skin. In, Kirkwood JM, Lotze MT, Yasko JM ed. Current Cancer Therapy, 2nd Ed.; Chapter 13, pp298-306; Churchill Livingston. Philadelphia, PA; 1996.

249. Lotze MT, Rubin JT. Biologic Therapeutics. In, Kirkwood JM, Lotze MT, Yasko JM ed. Current Cancer Therapy, 2nd Ed.; Chapter 4, pp66-86; Churchill Livingston. Philadelphia, PA; 1996.

250. Chang AE, Lotze MT, Pardoll DM. Mobilizing the body=s defenses against cancer.Patient Care 30:32-49, 1996.

251. Pappo I, Lotze MT. Immunotherapy of lung cancer. In Landreneau R, editor. Thoracic Surgery, in press.

252. Elder EM, Lotze MT, Whiteside TL. Methods for generation of genetically-modified fibroblasts for immunotherapy of cancer. In Methods in Molecular Biology: Gene Therapy Protocols, Ed. Paul Robbins, in press.

253. Tahara H, Lotze MT. Cancer gene therapy using cytokine genes. Submitted.

---

254. Maeurer M, Zitvogel L, Storkus WJ, Lotze MT. Human intestinal v) T-cells respond exclusively to SEB, but not to SEA. Natural Immunity 14:188-97, 1995.

255. Zitvogel L, Robbins PF, Clarke MR, Abe R, Davis CG, and Lotze MT. B7.1 costimulation markedly enhances IL-12 mediated antitumor immunity *in vivo*. Eur Jl Immun.26:1335-1341, 1996.

256. Berman RM, Suzuki T, Tahara H, Narula SK, Robbins PD, Lotze MT. Systemic administration of cellular Interleukin-10 (cIL-10) induces an effective, specific and long-lived immune response against established tumors in mice. J Immunology 157:231-238, 1996.

257. Maeurer MJ, Martin DM, Storkus WJ, Lotze MT. Evidence for shared T-cell receptor usage in cytolytic T-cells recognizing HLA-A2 presented antigens shared by melanoma and melanocytes. Immunology Today 16:603-4, 1995.

258. Maeurer MJ, Martin DM,Walter W, Liu Kaihong, Zitvogel L, Haluszczak C, Rabinowich H, Duquesnoy R, Storkus WJ, Lotze MT. Vä1+ T-lymphocytes obtained from colorectal cancer patients recognize tumor cells of epithelial origin. J. Exp. Med, 183:1681-96, 1996.

259. Lotze MT. Cytokines and the Treatment of Cancer (Chapter 199; pp199.1-199.25). The Handbook of Experimental Immunology, 5th Edition, Ed Weir, Herzenberg, Herzenberg, and Blackwell. Blackwell Science, Inc.; Cambridge, MA; 1996.

260. Mayordomo JI, Loftus DJ, Sakamoto H, Lotze MT, Storkus WJ, Appella E, DeLeo AB. Therapy of murine tumors with dendritic cells pulsed with p53 wild type and mutant sequence peptides. J Exp Med 183:1357-1365, 1996.

261. Farhood H, Storkus WJ, Lotze MT. IL-12 and IL-2 synergistically induce IL-10 mRNA and protein in activated human lymphocytes. Annals of the New York Academy of Sciences 1996;795.

262. Tahara H, Zitvogel L, Storkus WJ, Robbins PD, Lotze MT. Murine models of cancer cytokine gene therapy using IL-12. Annals of the New York Academy of Sciences 1996;795:275-283.

263. Zitvogel L, Mayordomo JI, Storkus WJ, Tahara H, Robbins PD, Finn O, Lotze MT. Specific active immunotherapy for breast carcinoma: genetically modified tumor vaccines and peptide pulsed dendritic cell-based adoptive therapy. Preclinical studies and clinical trials. Colloquim - Scandinavian Breast Group; October 11-14, 1995; Published by Bristol Myers Squibb.

264. Dookeran KA, Lotze MT, Sikora SS, Rao UNM. Pancreatic and periampullary carcinomas with epididymo-orchidic metastases. British Journal of Surgery 84:198-199, 1997.

265. Falo LD, Lotze MT. Cancer Vaccines, in Manual of Clinical Laboratory Immunology; 5th Edition. Chapter 133. Volume Editor: Everly Conway de Macario; Section Editor T. Whiteside. American Society for Microbiology Press, 1997.

266. Tzeng E, Shears LL, Lotze MT, Billiar TR. Gene Therapy. Current Prob. in Surgery 33:961-1052, 1996.

267. Wasserman K, Rosenstein MM, Lotze MT. Regression of established tumors by combined systemic administration of Interleukin 12 and local irradiation. Unpublished.

268. Galon J, Gauchat J-F, Villiers C, Mazieres N, Spagnoli R, Storkus W, Lotze MT, Bonnefoy J-Y, Fridman WH, Sautes C. Soluble Fcã receptors bind to CR3 (CD11b/CD18) and CR4 (CD11c/CD18) complement receptors. J Immunology 157:1184-92, 1996.

269. Maeurer MJ, Martin DM, Gollin S, Castelli C, Swaney W, Robbins PD, Storkus WJ, Lotze MT. Recurrent melanoma exhibiting downregulation of the peptide transporter protein, TAP-1 and antigen-loss of the immunodominant antigen MART-1/Melan-A recognized by CD8+ cytotoxic T-lymphocytes. Journal of Clinical Investigation 98:1633-41, 1996.

270. Dumanian G, Llull R, Ramasastry S, Lotze MT, Greco R, Edington H. Abodominal wall dehiscence with enterocutaneous fistula: Management with split thickness skin grafts.American Journal of Surgery 172:332-4, 1996.

271. Rubin JT, Brumfield A, Dookeran KA, Lotze MT. Oil-based , sustained-release cytokine preparations: vehicles for regional therapy of cancer and tumor. Unpublished.

272. Rubin JT, Brumfield A, Dookeran K, and Lotze MT. Oil-based, sustained-release interleukin-2: a vehicle for regional therapy of cancer and tumor vaccines. Unpublished.

273. Mertz PM, Corcoran ML, McCluskey KM, Zhang Y, Wong HL, Lotze MT, DeWitt DL, Wahl SM, Wahl LM.Suppression of prostaglandin H synthase-2 induction in human monocytes by *in vitro* or *in vivo* administration of interleukin 4. Cellular Immunology 173:252-260, 1996.

274. Dookeran KA, Lotze MT, Brumfield A, Rubin JT. Generation of multiple hepatic metastases in a rat tumour model. Unpublished.

275. Zitvogel L, Corey S, Tim, Maeurer MJ, Couderc B, Rybak ME, Lotze MT, Donnenberg A. Interleukin-4-induced apoptosis of a ää-T-cell leukemia: preclinical studies and *in vivo* application. Unpublished.

276. Goydos JS, Brumfield AM, Frezza E, Booth A, Lotze MT, Carty SE. Marked elevations in serum IL-6 levels in patients with cholangiocarcinoma. Annals of Surgery 227:398-404, 1998.

277. Goydos JS, Finn OJ, Lotze MT. A phase I trial of a synthetic mucin peptide vaccine. Induction of specific immune reactivity in patients with adenocarcinoma. J Surg Res 63:298-304, 1996.

278. Leder GH, Oppenheim M, Rosenstein M, Hoffman R, Lotze MT, Beger HG. NO fördert Tumorwachstum durch Lymphozytensuppression - Versuchs *in vivo* (NO supports tumor growth by suppression of lymphocytes - experiments *in vivo*. Unpublished.

279. Maeurer MJ, Lotze MT. Immune Responses to Melanoma Antigens. Chapter 32 (pp517-534) In Balch Charles M., Houghton Alan N., Sober Arthur J., Soong Seng-jaw. Cutaneous Melanoma, 3rd edition, Quality Medical Publishing, Inc. St. Louis, MO; 1998

280. Margolin K, Atkins M, Sparano J, Sosman J, Weiss G, Lotze M, Doroshow J, Mier J, O=Boyle K, Fisher R, Campbell E, Rubin J, Federighi D, Bursten S. Prospective randomized trial of lisofylline for the prevention of toxicities of high-dose interleukin-2 therapy in advanced renal cancer and malignant melanoma. Clinical Cancer Research 3:565-572, 1997.

281. Mayordomo JI, Zitvogel L, Tjandrawan T, Lotze MT, Storkus WJ. Dendritic cells presenting tumor peptide epitopes stimulate effective anti-tumor CTL *in vitro* and *in vivo*.In Immunology of Human Melanoma (Series: Biomedical and Health Research 12 (1996)), pp. 153-163.

282. Vokes EE, Figlin R, Hochster H, Lotze M, Rybak ME. A Phase II study of recombinant human interleukin 4 for advanced or recurrent non-small lung cancer. The Cancer Journal from Scientific American 4:46-51, 1998.

283. Atkins MB, Robertson MJ, Gordon M, Lotze MT, DeCoste M, DuBois JS, Ritz J, Sandler AB, Edington HD, Garzone PD, Mier JW, Canning CM, Battiato L, Tahara H, Sherman ML. Phase I

Evaluation of intravenous recombinant human interleukin-12 (rhIL-12) in patients with advanced malignancies. Clinical Cancer Research 1997 Mar; 3:409-417.

284. Tjandrawan T, Martin DM, Maeurer MJ, Castelli C, Lotze MT, Storkus WJ. Autologous human dendriphages pulsed with synthetic or natural tumor peptides elicit tumor-specific CTLs *in vitro*. J Immunother 21:149-157, 1998.

285. Shurin MR, Panharipande PP, Lotze MT. FLT3 receptor and ligand: Biology and potential clinical application. Unpublished.

286. Lotze MT. Introduction. Interleukin 12: Cellular and Molecular Immunology of an Important Regulatory Cytokine. NYAS 795:xiii-xix, 1996. Ed. Michael T. Lotze, Giorgio Trinchieri, Maurice K. Gately, and Stanley F. Wolf. New York Academy of Sciences, NY.

287. Lotze MT. Biology of Cancer Metastases: Lymphatic Dissemination. In Regional Therapy of Advanced Cancer, Ed. Lotze MT, Rubin JT; JB Lippincott, Philadelphia, 1996.

288. Maeurer MJ, Walter W, Martin D, Zitvogel L, Elder E, Storkus W, Lotze MT. Interleukin-7 (IL-7) in colorectal cancer: IL-7 is produced by cultured colorectal cancer cells and tissues from colorectal cancer. Scandinavian J Immunology,45:182-192, 1997.

289. Lotze MT,Zitvogel L, Campbell R, Robbins PD, Elder EM, Haluszczak C, Martin D, Whiteside TL, Storkus WJ, and Tahara H. Cancer Gene Therapy Using IL-12. NYAS 795:440-454, 1996. In Interleukin 12: Cellular and Molecular Immunology of an Important Regulatory Cytokine. Ed. Michael T. Lotze, Giorgio Trinchieri, Maurice K. Gately, and Stanley F. Wolf. New York Academy of Sciences, NY.

290. Kim SH, Ahn JS, Tahara H, Lotze MT, Robbins PD, Kim S. Retroviral-mediated IL-12 gene therapy fo advanced murine tumors: Implications for the immunotherapy of cancer. Unpublished.

291. Shurin MR, Pandharipande PP, Zorina TD, Haluszczak C, Subbotin VM, Hunter O, Brumfield A, Storkus W, Lotze MT. FLT3-Ligand-induced generation of functionally active dendritic cells in mice. Cellular Immunology 179:174-184, 1997.

292. Mayordomo JI, Zorina T, Storkus WJ, DeLeo AB, Zitvogel L, Lotze MT. Bone marrow-derived DCs serve as potent adjuvants for peptide-based antitumor vaccines. Stem Cells, 15:94-103, 1997.

293. Sikora SS, Kinzler D, Lotze MT. Peripheral nerve entrapment: an unusual adverse event with high-dose IL-2 therapy. Ann. Oncol 7:535, 1996.

294. Lotze MT, Shurin M, Davis I, Amoscato A, Storkus WJ. Dendritic cell based therapy of cancer. Adv Exp Med Biol 1997; 417:551-569.

295. Maeurer MJ, Lotze MT. Interleukin-7 (IL-7) knock out mice. Implications for lymphopoiesis and

organ-specific immunity. International Reviews of Immunology 1998;16(3-4):309-322.

296. Wilson Cara C, Tueting T, Ma DI, Haluszczak C, Lotze M, Storkus W. Activation of dendritic cells by surrogate T cell interactions leads to enhanced costimulation, secretion of TH1-associated cytokines, and CTL inductive capacity. In Procedings of the IV International Symposium on Dendritic Cells in Fundamental and Clinical Immunology (Vol 3). Ed. Paola Ricciardi-Castagnoli, Plenum Press, New York.

297. Bernhard H, Maeurer MJ, Jäger E, Wölfel, Schneider J, Karbach J, Seliger B, Huber C, Storkus WJ, Lotze MT, Meyer zum Büschenfelde K.-H., Knuth A. Recognition of human renal cell carcinoma and melanoma by HLA-A2-restricted cytotoxic T lymphocytes is mediated by shared peptide epitopes and up-regulated by Interferon-ã. Scand. J. Immunol 44:285-292, 1996.

298. Tuting T, Zorina T, Ma DI, Wilson CC, De Cesare CM, De Leo AB, Lotze MT, Storkus WJ. Development of dendritic cell-based genetic vaccines for cancer. Adv Exp Med Biol 1997;417:511-518.

299. Myers JN, Mank-Seymour A, Storkus W, Johnson CS, Lotze MT. SCCVII, a murine squamous cell carcinoma cell line that is a useful model for immunotherapy, Unpublished.

300. Myers JN, Mank-Seymour A, Zitvogel L, Storkus W, Clarke M, Johnson CS, Tahara H, Lotze MT. Interleukin-12 gene therapy prevents establishment of SCCVII squamous cell carcinomas, inhibits tumor growth, and elicits long term anti-tumor immunity in syngeneic C3H mice. Laryngoscope 108:261-268, 1998.

301. Couderc B, Zitvogel L, Doulin-Echinard V, Djennane L,Tahara H, Favre G, Lotze MT, Robbins PD. Enhancement of antitumor immunity by expression of CD70 (CD27L) or CD154(CD40L) costimulatory molecules on tumor cells.Cancer Gene Therapy 5:163-175, 1998.

302. Lotze MT, Zola H. CD30 Panel Report. Leucocyte Typing V: 6th International Human Leucocyte Differentiation Antigen Workshop Proceedings

303. Zitvogel L, Couderc B, Mayordomo JI, Robbins PD, Lotze MT, Storkus WJ. IL-12-engineered dendritic cells serve as effective tumor vaccine adjuvants *in vivo*. NYAS 795:284-293, 1996. In Interleukin 12: Cellular and Molecular Immunology of an Important Regulatory Cytokine. Ed. Michael T. Lotze, Giorgio Trinchieri, Maurice K. Gately, and Stanley F. Wolf. New York Academy of Sciences, NY.

304. Edington H, Lotze MT. Interleukin 7. Chapter 9; pp169-185 in The Cytokine Handbook, Second Edition; Editor, A. Thomson, Academic Press, Ltd; London; 1994.

305. Davis ID, Tahara H, Lotze MT. Cytokine Gene Therapy. in The Cytokine Handbook, Third Edition; Editor, A. Thomson, Academic Press, Ltd; London; 1998.

306 Tüting T, Storkus WJ, Lotze MT. Gene-based strategies for the immunotherapy of cancer. Molecular Medicine 75:478-491, 1997.

307. Zitvogel L, Le Cesne A, Cordier L, Lotze MT, Escudier B, Haddada H, Tursz T, Kourilsky F. specific active immunotherapy for cancer:Gene or dendritic cell-based tumor vaccines. In Sourcebook on Asbestos Diseases (Volume 13). George A and Barbara J. Peters, Eds.; The Michie Company/Butterworth and Company, Publishers, Inc.Borough Green, UK; 1996.

308. Goldman SA, Baker E, Weyent RJ, Clarke MR, Myers J, Lotze MT. Peritumoral CD1a-positive dendritic cells are associated with survival, recurrence and tumor stage in oral tongue squamous cell carcinoma. Archives of Otolaryngology Head and Neck Surgery 124:641-6, 1998.

309. Evans CH, Robbins PD, Ghivizzani SC, Herndon JH, Kang R, Bahnson AB, Barranger JA, Elder EM, Gay S, Tomaino MM, Wasko MC, Watkins SC, Whiteside TL, Glorioso JC, Lotze MT, Wright TM. Clinical trial to assess the safety, feasibility, and efficacy of transferring a potentially anti-arthritic cytokine gene to human joints with rheumatoid arthritis. Human Gene Therapy 7:1261-80, 1996.

310. Tüting T, DeLeo AB, Lotze MT, Storkus WJ. Genetically modified bone marrow-derived dendritic cells expressing tumor-associated viral or "self" antigens induce antitumor immunity *in vivo*. Eur. J. Immunology 27:2702-2707, 1997.

311. Tahara H, Kitagawa T, Iwazawa T, Lotze MT. Chapter 6. Gene transfection using particle bombardment. In Gene Therapy, Principles and Applications, Ed. Thomas Blankenstein. Birkhäuser Publishing Ltd.; pp93-102; Basel, Switzerland; 1999.

312. Maeurer MJ, Chan JH-W, Karbach J, Salter RD, Knuth A, Lotze MT, Storkus WJ. Amino acid substitution at position 97 in HLA-A2 segregate cytolysis from cytokine release in MART-1/Melan-A peptide AAGIGILTV-specific cytotoxic T lymphocytes. Eur. J. Immunol. 26:2613-2623, 1996.

313. Maeurer MJ, Edington H, Lotze MT. Interleukin 7. in The Cytokine Handbook, Third Edition; Editor, A. Thomson, Academic Press, Ltd; London;1999.

314. Lotze MT. Getting to the Source: Dendritic Cells as Therapeutic Reagents For The Treatment of Cancer Patients (Editorial). Annals of Surgery 226:1-5, 1997.

315. Tüting T, Baar J, Gambotto A, Davis ID, Storkus WJ, Zavodny P, Narula S, Tahara H, Robbins PD, Lotze MT. Interferon alpha gene therapy for cancer: retroviral transduction of fibroblasts and particle-mediated transfection of tumor cells are equally effective strategies for gene delivery in murine tumor models. Gene Therapy 4:1053-1060, 1997.

316. Shurin MR, Esche C, Peron J-M, Lotze MT. Anti-tumor activities of Interleukin-12 and mechanisms of action. Chemical Immunology 68:153-174, 1997.

317. Baar J, Lotze MT. Interferon-ã-inducible cytokine expression in plasmid transfected dendritic cells. Submitted for publication.

318. Vu HN, Jenkins FJ, Swerdlow SH, Locker J, Lotze MT. Pleural effusion as the presentation for primary effusion lymphoma. Surgery 123:589-591, 1998.

319. Amoscato AA, Prenowitz DA, Lotze MT. Rapid extracellular degradation of synthetic Class I peptides by human dendritic cells. J Immunology, 1998;161.

320. Tüting T, Wilson CC, Martin DM, Kasamon YL, Rowles J, Ma DI, Slingluff CL, Wagner SN, van der Bruggen P, Baar J, Lotze MT, Storkus WJ. Autologous human monocyte-derived dendritic cells genetically modifed to express melanoma antigens elicit primary cytotoxic T cell response *in vitro*: Enhancement by cotransfection of genes encoding the Th1-biasing cytokines IL-12 and IFN-á. J Immunol 1998;160:1139-1147.

321. Rosenberg SA, Blaese RM, Brenner MK, Deisseroth AB, Ledley FD, Lotze MT, Wilson JM, Nabel GJ, Cornetta K, Economou JS, Freeman SM, Riddell SR, Oldfield E, Gansbacher B, Dunbar C, Culver K, Heslop HE, Simons J, Wilmott RW, Tiberghien P, et al. Human gene marker/therapy clinical protocols. Human Gene Therapy 1996;7:2287-313.

322. Iwazawa, Chau G-Y, Mori T, Dookeran KA, Rubin JT, Watkins S, Robbins PD, Lotze MT, Tahara H. Potent antitumor effects of intra-arterial injection of fibroblasts genetically engineered to express IL-12 in liver metastasis model of rat: no additional benefit of using retroviral producer cell. Cancer Gene Ther 2001 Jan;8(1):17-22.

323. Gambotto A, Tüting T, McVey DL, Kovesdi I, Tahara H, Lotze MT, Robbins PD. Induction of anti-tumor immunity by direct intra-tumoral injection of a recombinant adenovirus vector expressing Interleukin 12. Cancer Gene Therapy 1999;6(1):45-53.

324. Antonysamy M, Lotze MT, Tahara H, Thomson A. Interleukins 16, 17, and 18 in The Cytokine Handbook, Third Edition; Editor, A. Thomson, Academic Press, Ltd; London;1999.

325. Osaki T, Péron J-M, Cai Q, Okamura H, Robbins PD, Kurimoto M, Lotze MT, Tahara H. Interferon-γ-inducing factor/Interleukin 18 Administration Mediates Interleukin 12 and Interferon-γ Independent Anti-tumor Effects. J Immunology 160:1742-1749, 1998.

326. Baar J, Storkus WJ, Lotze MT. Dendritic cells are potent mediators of tumour rejection. In Ciba-Geigy Bench-to-Bedside Series.

327. Storkus WJ, Tahara H, Lotze MT. Interleukin 12. In The Cytokine Handbook, Third Edition; Editor, A. Thomson, Academic Press, Ltd; London;1999.

328. Lotze MT. Future Directions for rIL-2 in Cancer: A Chronic Inflammatory Disorder. The Cancer Journal from Scientific American, 3:S106-109, 1997.

329. Lotze M, Hellerstedt B, Stolinski L, Tueting T, Wilson C, Storkus W, Tahara H, Kinzler D, Vu H, Rubin JT, Whiteside T, Elder E. Interleukin-2 (IL-2)and the role of dendritic cells (DCs) for cancer therapy. The Cancer Journal from Scientific American 3:S109-114, 1997.

330. Maeurer MJ, Lotze MT. Tumor recognition by the cellular immune system: new aspects of tumor immunology. International Reviews of Immunology 14:97-132, 1997.

331. Péron J.-M., Subbotin V, Esche C. Lotze MT, Shurin MR. Effective treatment of murine liver metastases: role of NK cells. Journal of Immunology 1998;161(11):6164-6170.

332. Doulin-Echinard V, Robbins PD, Lotze MT, Favre G, Couderc B. Enhancement of anti-tumor immunity by injection of fibroblasts genetically engineered to produce IL-12 and express CD70. Advances in Experimental Medicine and Biology 1998;451:353-357.

333. Fernandez Nadine, Duffour M-T, Perricaudet M, Lotze MT, Tursz T, Zitvogel L. Active specific immunotherapy for cancer: nucleic acids, peptides, whole native proteins, recombinant viruses, with dendritic cell adjuvants or whole tumor cell-based vaccines. Principles and future prospects. Cytokines and Molecular Therapy 4:53-66, 1998.

334. Dutcher JP, Atkins M, Fisher R, Weiss G, Margolin K, Aronson F, Sosman J, Lotze M, Gordon M, Logan T, Mier J. Interleukin-2-based therapy for metastatic renal cell cancer: The Cytokine Working Group experience, 1989-1997; Cancer Journal from Scientific American, 3:S73-78, 1997.

335. Robertson MJ, Cameron C, Atkins MB, Gordon MS, Lotze MT, Sherman M, Ritz J. Immunologic effects of Interleukin-12 administered as a bolus intravenous injection to patients with cancer. Clin Cancer Res 5:9-16, 1999.

337. Shurin MR, Esche C, Lotze MT. FLT3: Receptor and ligand. Biology and potential clinical application. Cytokine Growth Factor Rev 1998 Mar;9(1):37-48.

336. Okada H, Tahara H, Shurin MR, Attanucci J, Giezeman-Smits KM, Fellows WK, Lotze MT, Chambers WH, Bozik ME. Bone marrow-derived dendritic cells pulsed with a tumor-specific peptide elicit effective anti-tumor immunity against intracranial neoplasms. Int J Cancer 1998 Oct 5;78(2):196-201.

338. Farhood H, Campbell R, Shurin G, Hershberger P, Barksdale E, Watkins S, Lotze MT. Ubiquitous intracellular fas ligand expression in human and mouse tumor and normal cells. Unpublished.

339. Nishioka Y, Hirao M, Robbins PD, Lotze MT, Tahara H. Delivering Antigen Presenting Cells to the Site of Tumor Antigen: Induction of systemic and therapeutic antitumor immunity using intratumoral injection of dendritic cells genetically engineered to express Interleukin-12 (IL-12). Cancer Research 1999;59(16):4035-4041.

340. Shurin MR, Lokshin A, Shurin GV, Subbotin V, Hershberger P, Watkins SC, Esche C, Lotze MT. Tumors induce apoptosis of dendritic cells: another counterattack. Unpublished.

341. Maeurer MJ, Lotze MT. Interleukin-7 (IL-7) knockout mice. Implications for lyphopoiesis and organ-specific immunity. Intern. Rev. Immunol. 16:309-322, 1998.

342. Atkins MB, Lotze MT, Dutcher JP, Fisher R, Weiss G, Margolin K, Abrams J, Sznol M, Parkinson D, Hawkins M, Paradise C, Kunkel L, Rosenberg SA. High-dose recombinant interleukin-2 therapy for patients with metastatic melanoma: analysis of 270 patients treated from 1985-1993. Journal of Clinical Oncology 1999;17(7):2105.

343. Esche C, Subbotin VM, Hunter O, Wahl M, Peron M-M, Maliszewski C, Lotze MT, Shurin MR. Differential regulation of epidermal and dermal dendritic cells by IL-12 and Flt3 ligand. Journal of Investigative Dermatology 1999;113:1028-1032.

344. Finn OJ, Lotze MT. Third Keystone Symposium on Cellular Immunology and the Immunotherapy of Cancer. Introduction. J Immunother 21:114-118, 1998.

345. Lotze Michael T., Farhood Hassan, Wilson Cara C., Storkus Walter J. Section. V. DC Based Therapies; Chapter 25. Dendritic Cell Therapy of Cancer and HIV Infection in Dendritic Cells, edited by Lotze MT and Thomson A. Academic Press, London. 1998.

346. Wilson CC, Olson WC, Tüting T, Rinaldo CR, Lotze MT, Storkus WJ. HIV-1 specific cytotoxic

T-cell responses primed *in vitro* by blood derived dendritic cells and Th1-biasing cytokines. Journal of Immunology 1999;162(5):3070-3078.

347. Berman RM, Tahara H, Lotze MT. Viral Interleukin-10 (vIL-10), the human herpesvirus-4 (EBV) IL-10 homologue. Submitted

348. Berman RM, Tahara H, Satwant K. Narula, Lotze MT. Cellular mechanisms of anti-tumor immunity and immune memory generated by systemic administration of interleukin-10 to tumor-bearing mice. Submitted.

349. Campbell RL, Wright SE, Repasky EA, Watkins SC, Lotze MT. Early polar spectrin aggregation and uropod formation in CD45RA+ T-cell during primary activation by DCs. Submitted.

350. Lotze MT, Jaffe R. Section. IV. Dendritic Cells and Disease; Chapter 19. Cancer; In Dendritic Cells, edited by Lotze MT and Thomson A. Academic Press, London. 1998.

351. Esche C, Gambotto, A, Satoh Y, Gerein V, Robbins PD, Watkins SC, Lotze MT, Shurin MR. CD154 (CD40L) inhibits tumor-induced apoptosis in DCs and tumor growth. European Journal of Immunology 1999;29(7):2148-2155.

353. Satoh Y, Gambatto A, Esche C, Todo S, Lotze MT, Shurin MR. Dendritic cell-based IL-12 gene therapy in a model of colorectal cancer-derived hepatic metastases.. J Exp Ther Oncol 2002 Nov-Dec;2(6):337-49

354. Chikamatsu K, Nakano K, Storkus WJ, Appella E, Lotze MT, Whiteside TL, DeLeo AB. Generation of anti-p53 cytotoxic T lymphocytes from human peripheral blood using autologous dendritic cells. Clinical Cancer Research 1999;5(6):1281-1288.

355. Hashimoto W, Osaki T, Okamura H, Robbins PD, Kurimoto M, Nagata S, Lotze MT, Tahara H. Differential antitumor effects of administration of recombinant interleukin 18 (rIL-18) or rIL-12 are mediated by Fas-Fas ligand and perforin-induced tumor apoptosis, respectively. Journal of Immunology 1999;163(2):583-589.

356. Martincic K, Campbell R, Edwalds-Gilbert G, Souan L, Lotze MT, Milcarek C. Increase in the 64 kDa subunit of the polyadenylation/cleavage stimulatory factor (CstF-64) during the G0 to S-phase transition. PNAS 95:11095-11100, 1998.

357. Chi KH, Myers JN, Chow KC, Chan WK, Tsang YW, Chao Y, Yen SH, Lotze MT. Phase II trial of systemic recombinant IL-2 in the treatment of refractory nasopharyngeal carcinoma. Oncology 2001;60(2):110-5.

358. Peron JM, Esche C, Subbotin VM, Maliszewski C, Lotze MT, Shurin MR. FLT3-ligand administration inhibits liver metastases: role of NK cells. J Immunol 1998 Dec 1;161(11):6164-70.

359. Rao UN, Hanan SH, Lotze MT, Karakousis CP. Distant skin and soft tissue metastases from sarcomas. J Surg Oncol 1998 Oct;69(2):94-8.

360. Okada H, Attanucci J, Giezeman-Smits KM, Brissette-Storkus C, Fellows WK, Gambotto A, Pollack LF, Pogue-Geile K, Lotze MT, Bozik ME, Chambers WH. Immunization with an antigen identified by cytokine tumor vaccine-assisted SEREX (CAS) suppressed growth of the rat 9L glioma in vivo. Cancer Res 2001 Mar 15;61(6):2625-31

361. Giezeman-Smits KM, Okada H, Brisette-Storkus C, Villa LA, Attanucci J, Lotze MT, Pollack IF, Bozik ME, Chambers WH. Cytokine gene therapy of gliomas: induction of reactive CD4+ T cells by interleukin-4-transfected 9L gliosarcoma is essential for protective immunity. Cancer Res. 2000 May 1;60(9):2449-57.

362. McMasters KM, Sondak VK, Lotze MT, Ross MI. Recent advances in melanoma staging and therapy. Ann. Surg. Onc. 1999;6(5):467-475.

363. Campbell RL, Wright SE, Repasky EA, Watkins SC and Lotze MT. Specrin aggregation correlates with uropod formation: requirement for myosin motor activity and actin polymerization. Submitted.

364. Tahara H, Elder EM, Zitvogel LM, Storkus WJ, Robbins PD, Cai Q, Kinzler DM, Kirkwood J, Whiteside T, Lotze MT. Interleukin 12 (IL-12) gene therapy using direct injection of tumors with genetically engineered autologous fibroblasts: A Phase I Clinical Trial; submitted NEJM/

365. Shurin GV, Lotze MT, Barksdale EM. Human neuroblastoma cells induce apoptosis of T-cells: I. Role of FasL. *Nat Immun* 1998;16(5-6):263-74.

366. Zitvogel L, Tahara H, Mayordomo JI, Storkus WJ, Robbins PD, Elder EM, Whiteside TL, Lotze MT. Specific active immunottherapy for cancer: gene or dendritic cell-based tumor vaccines. Preclinical studies and clinical trials. In The Biotherapy of Cancers: From immunotherapy to gene therapy. Ed. S. Chouaib; Les Editions, INSERM, Paris, 1998 pp 475-490.

367. Odoux C, Lotze MT, Siegfried JM, Luketich JD, Weigel TL. Paclitaxel induces apoptotic death and activates caspase-3 in human lung cancer: is it really Fas dependent? Submitted for publication.

368. Takayama T, Nishioka Y, Lu L, Lotze MT, Tahara H, Thomson AW. Retroviral delivery of viral interleukin-10 into myeloid dendritic cells markedly inhibits their allostimulatory activity and promotes the induction of T-cell hyporesponsiveness.Transplantation 1998 Dec 27;66(12):1567-74.

369. Hirao M, Nishioka Y, Watkins SC, Robbins PD, Lotze MT, Tahara H. Tumor specific cellular immune response induced with intratumoral injection of immature dendritic cells, submitted.

370. Okada H, Giezeman-Smits KM, Tahara H, Attanucci J, Fellows WK, Lotze MT, Chambers WH, ME. Effective cytokine gene therapy against an intracranial glioma using a retrovirally transduced IL-4 plus HSVtk tumor vaccine. Gene Therapy 1999;6(2):219-226.

371. Okada H, Lotze MT. Interleukin 4: Clinical Applications in Rosenberg SA Principles and Practice of the Biologic Therapy of Cancer; 3rd Edition. pp93-103; Lippincott Williams Wilkins, Philadelphia; 2000.

372. Amoscato AA, Matsko CM, Rabinowich H, Lotze MT. Mitochondrial phosphatidylglycerol cardiolipi and ceramide alterations occur as very early events during T-cell apoptosis. Submitted.

373. Rosenberg SA, Blaese RM, Brenner MK, Deisseroth AB, Ledley FD, Lotze MT, Wilson JM, Nabel GJ, Cornetta K, Economou JS, Freeman SM, Riddell SR, Oldfield E, Gansbacher B, Dunbar C, Walter RE, Schuening FG, Roth JA, Crystal RG, Welsh MJ, Culver K, Keslop HE, Simons J, Wilmott RW, Habib NA. Human gene marker/therapy clinical protocols. Human Gene Therapy 1999;10(18):3067-3123.

374. Osaki T, Hashimoto W, Gambotto A, Okamura H, Robbins PD, Kurimoto M, Lotze MT, Tahara H. Potent antitumor effects mediated by local expression of the mature form of the interferon-gamma inducing factor, interleukin-18 (IL-18). Gene Therapy 1999;6(5):808-815.

375. Lotze MT. In Vivo Veritas. Clinical Immunology 1999;93(1):1-4.

376. Ohm JE, Shurin MR, Esche C, Lotze MT, Carbone DP, Gabrilovich DI. Effect of vascular endothelial growth factor and FLT3 ligand on dendritic cell generation *in vivo*. Journal of Immunology 199;163(6):3260-3268.

377. Esche C, Lokshin A, Shurin GV, Gastman BR, Rabinowich H, Watkins SC, Lotze MT, Shurin MR. Tumor's other immune targets: dendritic cells. Journal of Leukocyte Biology 1999;66(2):336-344.

378. Lotze MT. Not just an antigen-presenting cell: 5th international symposium on dendritic cells in fundamental and clinical immunology. Journal of Leukocyte Biology 1999;66(2):195-200.

379. Tuting T, Gambotto A, DeLeo A, Lotze MT, Robbins PD, Storkus WJ. Induction of tumor antigen-specific immunity using plasmid DNA immunization in mice. Cancer Gene Therapy 1999;6(1):73-80.

380. Thomas RL, Lotze MT, Amoscato AA. Qualitative whole cell lipid analysis of neoplastic cell lines via electrospray ionization tandem mass spectrometry. Submitted for publication.

381. Esche C, Makarenkova VP, Kost NV, Lotze MT, Zozulya AA, Shurin MR. Murine dendritic cells express functional delta-type opioid receptors. Annals of the New York Academy of Sciences 1999;885:387-390.

384. Shurin MR, Lu L, Kalinski P, Stewart-Akers AM, Lotze MT. Th1/Th2 balance in cancer, transplantation and pregnancy. Springer Semin Immunopathol. 1999;21(3):339-59.

382. Hiroishi K, Tuting T, Tahara H, Lotze MT Interferon-alpha gene therapy in combination with CD80 transduction reduces tumorigenicity and growth of established tumor in poorly immunogenic tumor models. Gene Ther. 1999 Dec;6(12):1988-94.

385. Rosenberg SA, Blaese RM, Brenner MK, Deisseroth AB, Ledley FD, Lotze MT, Wilson JM, Nabel GJ, Cornetta K, Economou JS, Freeman SM, Riddell SR, Oldfield E, Gansbacher B, Dunbar C, Walker RE, Schuening FG, Roth JA, Crystal RG, Welsh MJ, Culver K, Heslop HE, Simons J, Wilmott RW, Habib NA, et al. Human gene marker/therapy clinical protocols. Hum Gene Ther. 1999 Dec 10;10(18):3067-123.

386 Esche C, Subbotin VM, Hunter O, Peron JM, Maliszewski C, Lotze MT, Shurin MR. Differential regulation of epidermal and dermal dendritic cells by IL-12 and Flt3 ligand. J Invest Dermatol. 1999 Dec;113(6):1028-32.

387. Atkins MB, Lotze MT, Dutcher JP, Fisher RI, Weiss G, Margolin K, Abrams J, Sznol M, Parkinson D, Hawkins M, Paradise C, Kunkel L, Rosenberg SA. High-Dose Recombinant Interleukin 2 Therapy for Patients With Metastatic Melanoma: Analysis of 270 Patients Treated Between 1985 and 1993.J Clin Oncol. 1999 Jul;17(7):2105.

388. Thomas RL Jr, Matsko CM, Lotze MT, Amoscato AA Mass spectrometric identification of increased C16 ceramide levels during apoptosis. J Biol Chem. 1999 Oct 22;274(43):30580-8.

389. Osaki T, Hashimoto W, Gambotto A, Okamura H, Robbins PD, Kurimoto M, Lotze MT, Tahara H. Potent antitumor effects mediated by local expression of the mature form of the interferon-gamma inducing factor, interleukin-18 (IL-18).Gene Ther. 1999 May;6(5):808-15.

390. Ohm JE, Shurin MR, Esche C, Lotze MT, Carbone DP, Gabrilovich DI. Effect of vascular endothelial growth factor and FLT3 ligand on dendritic cell generation in vivo.J Immunol. 1999 Sep 15;163(6):3260-8.

391. Nishioka Y, Hirao M, Robbins PD, Lotze MT, Tahara H. Induction of systemic and therapeutic antitumor immunity using intratumoral injection of dendritic cells genetically modified to express interleukin 12. Cancer Res. 1999 Aug 15;59(16):4035-41.

392. Saito T, Dworacki G, Gooding W, Lotze MT, Whiteside TL. Spontaneous apoptosis of CD8+ T lymphocytes in peripheral blood of patients with advanced melanoma. Clinical Cancer Research 6:1351-1364, 2000.

393. Lotze MT Not just an antigen-presenting cell: 5th international symposium on dendritic cells in fundamental and clinical immunology. J Leukoc Biol. 1999 Aug;66(2):195-200.

394. Son YI, Dallal RM, Mailliard RB, Egawa S, Jonak ZL, Lotze MT. Interleukin-18 (IL-18) synergizes with IL-2 to enhance cytotoxicity, interferon-gamma production, and expansion of natural killer cells. Cancer Res 2001 Feb 1;61(3):884-8

395. Weigel TL, Lotze MT, Kim PK, Amoscato AA, Luketich JD, Odoux C. Paclitaxel-induced apoptosis in non-small cell lung cancer cell lines is associated with increased caspase-3 activity. J Thorac Cardiovasc Surg. 2000 Apr;119(4):795-803.

396. Okada H, Pollack IF, Lotze MT, Lunsford LD, Kondziolka D, Lieberman F, Schiff D, Attanucci J, Edington H, Chambers W, Robbins P, Baar J, Kinzler D, Whiteside T, Elder E. Gene therapy of malignant gliomas: a phase I study of IL-4-HSV-TK gene-modified autologous tumor to elicit an immune response. Hum Gene Ther. 2000 Mar 1;11(4):637-53.

397. Lotze MT, Shurin M, Esche C, Tahara H, Storkus W, Kirkwood JM, Whiteside TL, Elder EM, Okada H, Robbins P. Interleukin-2: developing additional cytokine gene therapies using fibroblasts or DCs to enhance tumor immunity. Cancer J Sci Am. 2000 6 Suppl 1:S61-6.

398. Lotze MT. The future role of interleukin-2 in cancer therapy. Cancer J Sci Am. 2000 Feb;6 Suppl 1:S58-60.

399. Hiroishi K, Tuting T, Lotze MT IFN-alpha-expressing tumor cells enhance generation and promotes survival of tumor-specific CTLs. J Immunol. 2000 Jan 15;164(2):567-72

400. Agha-Mohammadi S, Lotze MT. Regulatable systems: applications in gene therapy and replicating viruses. J Clin Invest. 2000 May;105(9):1177-83. Review.

401. Esche C, Shurin MR, Lotze MT. IL-12R. In Cytokines, Ed Oppenheim J et al Academic Press, London; 2000.

402. Esche C, Shurin MR, Lotze MT. IL-12. In Cytokines, Ed Oppenheim J et al Academic Press, London; 2000.

403. Lotze MT, Dallal RM, Kirkwood JM, Flickinger JC. Cutaneous Melanoma. Chapter 42.2. In Principles and Practice of Oncology, Ed. DeVita V, Hellman S, Rosenberg S. Lippincott-Raven Press, Philadelphia; 2001.

405. Dallal RM, Mailliard R, Lotze MT. Dendritic Cell Vaccines. In Principles and Practice of Biologic Therapy, Ed Rosenberg SA; pp 705-721; Lippincott Williams and Wilkins Press, Philadelphia; 2001.

406. Esche C, Shurin MR, Lotze MT. The use of dendritic cells for cancer vaccination. Current Opinion in Molecular Therapeutics 1999;1(1):72-81.

407. Lotze MT, Odoux C, Luketich J. Editorial Comment: Gene Therapy of Lung Cancer: And Miles to Go Before We Reap. In press, Lung Cancer 2000.

408. Dallal RM, Lotze MT. The Dendritic Cell and Human Cancer Vaccines; *Curr Opin Immunol* 2000 Oct;12(5):583-8

409. Tanaka F, Hirao M, Lotze MT. Delivery of dendritic cells to sites of immune reactivity. Clinical Immunology Newsletter 19:128-131, 1999.

410. Agha-Mohammadi S, Lotze MT. Immunomodulation of cancer: potential use of selectively replicating agents. J Clin Invest. 2000 May;105(9):1173-6.

411. Mailliard RB, Lotze MT. Dendritic Cells Prolong Tumor Specific T-cell Survival and Effector Function after interaction with tumor targets. Clin Cancer Research, Res 2001 Mar;7(3 Suppl):980s-988s

412. Tahara H, Lotze MT. Interleukin 12: Clinical Applications. in Rosenberg SA Principles and Practice of the Biologic Therapy of Cancer; 3rd Edition. pp103-112; Lippincott Williams Wilkins, Philadelphia; 2000.

413. Kikly K, Lotze MT. DCs and wound healing. In Lotze MT and Thomson AW. Dendritic Cells, 2nd Edition. Academic Press, London; 2001.

414. Mailliard RB, Dallal RM, Son YI, Lotze MT. Dendritic cells promote T-cell survival or death depending upon their maturation state and presentation of antigen. Immunol Invest. 2000 May;29(2):177-85.

415. Ranieri E, Kierstead LS, Zarour H, Kirkwood JM, Lotze MT, Whiteside T, Storkus WJ. Dendritic cell/peptide cancer vaccines: clinical responsiveness and epitope spreading. Immunol Invest. 2000 May;29(2):121-5.

416. Hirao M, Onai N, Hiroishi K, Watkins SC, Matsushima K, Robbins PD, Lotze MT, Tahara H. CC chemokine receptor-7 on dendritic cells is induced after interaction with apoptotic tumor cells: critical role in migration from the tumor site to draining lymph nodes. Cancer Res. 2000 Apr 15;60(8):2209-17.

417. Rosenberg SA, Blaese RM, Brenner MK, Deisseroth AB, Ledley FD, Lotze MT, Wilson JM, Nabel GJ, Cornetta K, Economou JS, Freeman SM, Riddell SR, Brenner M, Oldfield E, Gansbacher B, Dunbar C, Walker RE, Schuening FG, Roth JA, Crystal RG, Welsh MJ, Culver K, Heslop HE, Simons J, Wilmott RW, Boucher RC. Human gene marker/therapy clinical protocols. Hum Gene Ther. 2000 Apr 10;11(6):919-79.

418. Tanaka F, Hashimoto W, Okamura H, Robbins PD, Lotze MT, Tahara H
Rapid generation of potent and tumor-specific cytotoxic T lymphocytes by interleukin 18 using dendritic cells and natural killer cells. *Cancer Res* 2000 Sep 1;60(17):4838-44

419. Hashimoto W, Tanaka F, Robbins PD, Taniguchi M, Okamura H, Lotze MT, Tahara H. Natural killer, but not natural killer T, cells play a necessary role in the promotion of an innate antitumor response induced by IL-18. Int J Cancer 2003 Feb 1;103(4):508-13

420. Agha-Mohammadi S, Etemad A, Lotze MT. Tet-on or tet-off: Uninduced reverse tTA displays partial affinity for tet operators, limiting efficiency of the Tet-on system. J Biological Chemistry, submitted.

421. Agha-Mohammadi, Lotze MT. Spacially optimized tet-operator positioning allows markedly enhanced efficiency of tet-regulated gene expression. Nature Biotechnology, submitted.

422. Kalinski P, Lotze MT, Kapsenberg ML. Dendritic cell-related immunoregulatory factors. Dendritic Cells; Ed. 2; Lotze, MT and Thomson AW, editors; Academic Press, London; 2001.

423. Davis ID and Lotze MT: Melanoma peptide clinical trials. In: Peptide-based cancer vaccines. Kast WM (ed), Eurekah.com, Austin,TX,USA. 215-236,2000.

424. Eto M, Bennouna J, Hunter OC, Lotze MT, Amoscato AA. Prostate cancer C16 apoptosis associated ceramide (CAAC) accumulates following androgen ablation. Science, submitted.

425. Shurin GV, Lotze MT, Barksdale EM Neuroblastoma inhibits dendritic cell differentiation and function. *Curr Surg* 2000 Nov;57(6):637.

426. Esche C, Cai Q, Peron JM, Hunter O, Subbotin VM, Lotze MT, Shurin MR Interleukin-12 and Flt3 ligand differentially promote dendropoiesis in vivo. *Eur J Immunol* 2000 Sep;30(9):2565-75

427. Dutcher JP, Logan T, Gordon M, Sosman J, Weiss G, Margolin K, Plasse T, Mier J, Lotze M, Clark J, Atkins M. Phase II trial of interleukin 2, interferon alpha, and 5-fluorouracil in metastatic renal cell cancer: a cytokine working group study. *Clin Cancer Res* 2000 Sep;6(9):3442-50.

428. Pirtskhalaishvili G, Shurin GV, Esche C, Cai Q, Salup RR, Bykovskaia SN, Lotze MT, Shurin MR. Cytokine-mediated protection of human dendritic cells from prostate cancer-induced apoptosis is regulated by the Bcl-2 family of proteins. *Br J Cancer* 2000 Aug;83(4):506-13

429. Mailliard R, Egawa S-I, Cai Q, Wankowicz-Kalinska A, Lotze MT, Kapsenberg ML, Storkus WJ, Kalinski WJ. Complementary DC-activating function of $CD8^+$ and $CD4^+$ T cells: Helper

role of $CD8^+$ T cells in the development of T Helper Type 1 Responses. J Exp Med. 2002 Feb 18;195(4):473-483.

430. Sanjay Kumar, Charles Hanning, Michael Brigham-Burke, David Rieman, Ruth Lehr, Sanjay Khandekar, Robert Kirkpatrick, Gilbert Scott, John C Lee, Maxine Gowen, Frank Lynch, Michael Lotze Interleukin-1 homologue H4 (IL-1H4/IL-1F7) is processed by caspase-1, binds to the IL-18 receptor but does not induce IFN-γ production. Cytokine 2002 Apr 21;18(2):61-71

431. Okada H, Villa L, Attanucci J, Erff M, Fellows WK, Lotze MT, Pollack IF, Chambers WH Cytokine gene therapy of gliomas: effective induction of therapeutic immunity to intracranial tumors by peripheral immunization with interleukin-4 transduced glioma cells. Gene Ther 2001 Aug;8(15):1157-66.

432. Flaherty LE, Atkins M, Sosman J, Weiss G, Clark JI, Margolin K, Dutcher J, Gordon MS, Lotze M, Mier J, Sorokin P, Fisher RI, Appel C, Du W.Outpatient biochemotherapy with interleukin-2 and interferon alfa-2b in patients with metastatic malignant melanoma: results of two phase II cytokine working group trials. J Clin Oncol 2001 Jul 1;19(13):3194-202

433. Kang WK, Park C, Yoon HL, Kim WS, Yoon SS, Lee MH, Park K, Kim K, Jeong HS, Kim JA, Nam SJ, Yang JH, Son YI, Baek CH, Han J, Ree HJ, Lee ES, Kim SH, Kim DW, Ahn YC, Huh SJ, Choe YH, Lee JH, Park MH, Kong GS, Park EY, Kang YK, Bang YJ, Paik NS, Lee SN, Kim SH, Kim S, Robbins PD, Tahara H, Lotze MT, Park CH. Interleukin 12 gene therapy of cancer by peritumoral injection of transduced autologous fibroblasts: outcome of a phase I study. Hum Gene Ther 2001 Apr 10;12(6):671-84.

434. Dallal RM, Lotze MT. Immunotherapy of metastasis. Surg Oncol Clin N Am 2001 Apr;10(2):433-47, xi.

435. Dworacki G, Meidenbauer N, Kuss I, Hoffmann TK, Gooding W, Lotze M, Whiteside TL. Decreased zeta chain expression and apoptosis in CD3+ peripheral blood T lymphocytes of patients with melanoma. Clin Cancer Res 2001 Mar;7(3 Suppl):947s-957s.

436. Finn OJ, Lotze MT. A decade in the life of tumor immunology. Clin Cancer Res 2001 Mar;7(3 Suppl):759s-760s.

437. Shurin GV, Shurin MR, Bykovskaia S, Shogan J, Lotze MT, Barksdale EM Jr.Neuroblastoma-derived gangliosides inhibit DC generation and function. Cancer Res 2001 Jan 1;61(1):363-9.

438. Uchibayashi N, Robbins PD, Lotze MT, Tahara H. Immature dendritic cells capturing exogenous antigens, generated ex vivo quantitatively from CD34+ blood progenitors cultured with GM-CSF and Flt-3 ligand. Submited for publication (Experimental Hematology).

439. Dallal RM, Christakos P, Lee K, Egawa S, Son Y-I, Lotze MT. Paucity of dendritic cells in pancreatic cancer. Surgery. 2002 Feb;131(2):135-8.

440. Matsko CM, Hunter OC, Rabinowich H, Lotze MT, Amoscato AA. Mitochondrial lipid alterations during fas- and radiation-induced apoptosis.Biochem Biophys Res Commun. 2001 Oct 12;287(5):1112-20.

441. Son YI, Mailliard RB, Watkins SC, Lotze MT.Dendritic cells pulsed with apoptotic squamous cell carcinoma have anti-tumor effects when combined with interleukin-2. Laryngoscope. 2001 Aug;111(8):1472-8.

442. Fujii Si, Shimizu K, Shimizu T, Lotze MT. Interleukin-10 promotes the maintenance of antitumor CD8(+) T-cell effector function in situ. Blood. 2001 Oct 1;98(7):2143-51.

443. Kanto T, Kalinski P, Hunter OC, Lotze MT, Amoscato AA.Ceramide mediates tumor-induced dendritic cell apoptosis. J Immunol. 2001 Oct 1;167(7):3773-84.

444. Shimizu T, Berhanu A, Redlinger RE Jr, Watkins S, Lotze MT, Barksdale EM Jr.Interleukin-12 transduced DCs induce regression of established murine neuroblastoma. J Pediatr Surg. 2001 Aug;36(8):1285-92.

445. Gao W, Kumar S, Lotze MT, Hanning C, Robbins PD, Gambotto A. Innate immunity mediated by the interleukin-1 homologue 4 (IL-1H4/IL-1F7) proinflammatory cytokine induces IL-12 dependent adaptive and profound antitumor immunity. J Immunol 2003 Jan 1;170(1):107-13.

446. Lotze MT, Thomson AW. In the medium is the message: cytokines and dendrikines regulate immune reactivity. Nature Reviews Immunology, in press.

447. Okada H, Lotze MT. Interleukin 4; Chapter 23; Cytokine Handbook 4E. Thomson AW; Lotze MT. Editors. Academic Press, London. 2003.

448. Kalinski P, Storkus WJ, Thomson AW, Lotze MT. Interleukin 12; Cytokine Handbook 4E. Thomson AW; Lotze MT. Editors. Academic Press, London. 2003.

449. Lotze MT, Tahara H, Okamura H. Interleukin-18 as a novel, distinct and distant member of the IL-1 family promoting development of the adaptive immune response. J Immunother. 25:S1-S3, 2002.

450. Jonak ZL, Trulli S, Maier C, McCabe FL, Kirkpatrick R, Johanson K, Ho YS, Elefante L, Chen Y-J, Herzyk D, Lotze MT, Johnson RK High-dose recombinanat Interleukin-18 induces an effective Th1 immune responset to murine MOPC-315 plasmacytoma. J Immunotherapy 25: S20-S27; 2002.

451. Odoux C, Albers A, Amoscato AA, Lotze MT, Wong MK. TRAIL, FasL and a blocking anti-DR5 antibody augment paclitaxel-induced apoptosis in human non-small-cell lung cancer. Int J Cancer 2002 Feb 1;97(4):458-65.

452. Agha-Mohammadi S, Lotze MT. Cytokine gene therapy. In Volume 2, Encyclopedia of Cancer;

Elsevier Science, 2002.

453. Shimamura H, Cumberland R, Hiroishi K, Watkins SC, Lotze MT, Baar J.Murine Dendritic Cell-Induced Tumor Apoptosis is Partially Mediated by Nitric Oxide. J Immunother 2002 May-Jun;25(3):226-34.

454. Lotze MT, Kost T. Viruses as Gene Delivery Vectors: Application to Gene Function, Target Validation and Assay Development. Cancer Gene Therapy, 2002 Aug;9(8):692-9.

455. Lotze MT, Kalinski P. Interleukin 12 Family [IL-12, IL-12RA, IL-23, IL-27]. In Encyclopedia of Hormones; Elsevier Science, 2003.

456. Lotze MT, Hamilton JA. Macrophage Colony Stimulating Factor [M-CSF]. In Cytokine Handbook, 4th Edition; Ed. Thomson AW, Lotze MT. Academic Press London. 2003

457. Haruki Okamura*, Hiroko Tsutsui, Michael T. Lotze+, Shin-ichiro Kashiwamura, Haruyasu Ueda, Tomohiro Yoshimoto, and Kenji Nakanishi. Interleukin 18 [IL-18] or the Interleukin-1 Family 4 [IL1-F4] Cytokine. In Cytokine Handbook, 4th Edition; Ed. Thomson AW, Lotze MT. Academic Press London. 2003

458. Numasaki M, Fukushi JI, Ono M, Narula SK, Zavodny PJ, Kudo T, Robbins PD, Tahara H, Lotze MT. Interleukin-17 promotes angiogenesis and tumor growth. Blood. 2003 Apr 1;101(7):2620-7.

459. Redlinger RE Jr, Mailliard RB, Lotze MT, Barksdale EM Jr.Synergistic interleukin-18 and low-dose interleukin-2 promote regression of established murine neuroblastoma in vivo. J Pediatr Surg 2003 Mar;38(3):301-7; discussion 301-7.

460. Lotze MT. The critical need for cancer biometrics: quantitative, reproducible measures of cancer to define response to therapy. Curr Opin Investig Drugs. 2003 Jun;4(6):649-51.

461. Eto M, Bennouna J, Hunter OC, Hershberger PA, Kanto T, Johnson CS, Lotze MT, Amoscato AA. C16 ceramide accumulates following androgen ablation in LNCaP prostate cancer cells. Prostate. 2003 Sep 15;57(1):66-79.

462. Son YI, Dallal RM, Lotze MT. Combined treatment with interleukin-18 and low-dose interleukin-2 induced regression of a murine sarcoma and memory response. J Immunother. 2003 May-Jun;26(3):234-40.

463. Fujii S-I, Lotze MT. IL-10 effects on innate immunity. In Marincola, FM, Interleukin 10; in press.

464. Kitagawa T, Iwazawa T, Robbins PD, Lotze MT, Tahara H. Advantages And Limitations Of Particle-Mediated Transfection (Gene Gun) In Cancer Immuno-Gene Therapy Using IL-10, IL-

12 Or B7-1 In Murine Tumor Models. J Gene Med. 2003 Nov;5(11):958-65.

465. Lotze MT, DeMarco RA. Dealing with Death: HMGB1 As a Novel Target for Cancer Therapy. Current Opinion in Investigational Drugs 2003 4(12):1405-1409.

466. Lotze MT, Rees RC. Identifying biomarkers and surrogates of tumors (cancer biometrics): correlation with immunotherapies and immune cells. Cancer Immunology, Immunotherapy, 2004 Mar;53(3):256-61.

467. Lotze MT, Papamichail M. A primer on cancer immunology and immunotherapy. Cancer Immunol Immunother. 2004 Mar;53(3):135-8.

468. Vakkila J, Lotze MT. Inflammation and necrosis promote tumour growth. *Nature Reviews Immunology* 4:641-647, 2004.

469. Lotze MT. Inflammation, necrosis, and cancer. In Cancer and Inflammation; edited by Doug Morgan, Ulf Forssmann, and Marian Nakada; Birkhauser Publishing, Basel Switzerland; pp. 189-196, 2004.

470. Vakkila J, Lotze MT, Riga C, Jaffe R. A basis for distinguishing cultured dendritic cells and macrophages in cytospins and fixed sections. Pediatr Dev Pathol. 2005 Jan-Feb;8(1):43-51.

471. Numasaki M, Lotze MT, Sasaki H. Interleukin-17 augments tumor necrosis factor-alpha-induced elaboration of proangiogenic factors from fibroblasts. Immunol Lett. 2004 Apr 30;93(1):39-43.

472. Lotze MT, Herberman RB. Cancer as a chronic inflammatory disease: role of immunotherapy. In Cancer and Inflammation; edited by Doug Morgan, Ulf Forssmann, and Marian Nakada; Birkhauser Publishing, Basel Switzerland; pp 21-51, 2004.

473. Demarco RA, Fink MP, Lotze MT. Monocytes promote Natural Killer Cell Interferon Gamma Production in Response to the Endogenous Danger Signal HMGB1. Molecular Immunology 42(4), February 2005, 433-444.

474. Vakkila J, DeMarco RA, Lotze MT. Imaging analysis of STAT1 and NFκB in Single Dendritic Cells. Journal of Immunological Methods 294(1-2) November 2004, 123-134.

475. Hauskrecht, M. R. Pelikan, D. Malehorn, W.L. Bigbee, M.T. Lotze, H.J. Zeh, III, D.C. Whitcomb, J. Lyons-Weiler. 2004. Feature Selection for Classification of SELDI-TOF-MS Proteomic Profiles. Applied Bioinformatics, in press.

476. Agha-Mohammadi S, O'Malley M, Etemad A, Wang Z, Xiao X, Lotze MT. Second-generation tetracycline-regulatable promoter: repositioned tet operator elements optimize transactivator synergy while shorter minimal promoter offers tight basal leakiness. J Gene Med. 2004

Jul;6(7):817-28.

477. Okada H, Lotze MT. Cytokines and Cancer Vaccines. In Caliguiri M, Lotze MT; editors; *Cytokines and Cancer*; in press 2005.

478. Zeh HJ, Lotze MT. Addicted to Death: Invasive Cancer and the Immune Response to Unscheduled Cell Death; J Immunother. 2005 Jan-Feb;28(1):1-9.

479. Lotze MT, Tracey KJ. High-mobility group box 1 protein (HMGB1): nuclear weapon in the immune arsenal. Nat Rev Immunol. 2005 Apr;5(4):331-42.

480. Tsung A, Sahai R, Tanaka H, Nakao A, Fink MP, Lotze MT, Yang H, Li J, Tracey KJ, Geller DA, Billiar TR. The nuclear factor HMGB1 mediates hepatic injury after murine liver ischemia-reperfusion. J Exp Med. 2005 Apr 4;201(7):1135-4.

481. Lotze MT, Wang E, Marincola FM, Hanna N, Bugelski PJ, Burns CA, Coukos G, Damle N, Godfrey TE, Howell WM, Panelli MC, Perricone MA, Petricoin EF, Sauter G, Scheibenbogen C, Shivers SC, Taylor DL, Weinstein JN, Whiteside TL. Workshop on cancer biometrics: identifying biomarkers and surrogates of cancer in patients. J Immunother. 2005 Mar-Apr;28(2):79-119.

482. Fujii S-I, Lotze MT. The IL-10 extended family of cytokines. In Caliguiri MC, Lotze MT. Cytokines and Cancer; Humana Press; in press.

483. Chang AE, Lotze MT, Slingluff CL, Sondak VK. Update on Immunotherapy in Cancer Treatment. Contemporary Surgery, in press.

484. Kalinski P, Mailliard RB, Giermasz A, Zeh HJ, Basse P, Bartlett DL, Kirkwood JM, Lotze MT, Herberman RB. NK-DC cross-talk in cancer immunotherapy. Exp.Opin Biol Ther. 2005 5:1303-15.

485. Guo ZS, Naik A, O'Malley ME, Popovic P, Demarco R, Hu Y, Yin X, Yang S, Zeh HJ, Moss B, Lotze MT, Bartlett DL. The enhanced tumor selectivity of an oncolytic vaccinia lacking the host range and antiapoptosis genes SPI-1 and SPI-2. Cancer Res. 2005 Nov 1;65(21):9991-8.

486. Takahashi H, Numasaki M, Lotze MT, Sasaki H. Interleukin-17 enhances bFGF-, HGF- and VEGF-induced growth of vascular endothelial cells. Immunol Lett. 2005 May 15;98:189-93.

487. Tsung A, Hoffman RA, Izuishi K, Critchlow ND, Nakao A, Chan MH, Lotze MT, Geller DA, Billiar TR. Hepatic Ischemia/Reperfusion Injury Involves Functional TLR4 Signaling in Nonparenchymal Cells. J Immunol. 2005 Dec 1;175(11):7661-8.

488. Lotze MT. Cancer Gene Therapy: The power of negative thinking. Gene Ther. 2006

Feb;13(3):191-3.

489. Vakkila J, Jaffe R, Michelow M, Lotze MT. Pediatric cancers are infiltrated predominantly by macrophages and have a paucity of dendritic cells. Clinical Cancer Research, 2006 Apr 1;12(7):2049-54.

490. Dong XD, Popovic P, Lotze MT, DeMarco RA, Watkins S, Ito N, Winikoff S, Bartlett DL, Zeh HJ. Sequestration of HMGB1 in the nucleus of dying tumor cells following treatment with the platinating agent oxaliplatin. Submitted for publication.

491. Montag DT, Lotze MT. Successful simultaneous measurement of cell membrane and cytokine induced phosphorylation pathways [CIPP] in human peripheral blood mononuclear cells. J Immunol Methods. 2006 Jun 30;313(1-2):48-60. Epub 2006 May 2.

492. Hauskrecht M, Pelikan R, Malehorn DE, Bigbee WL, Lotze MT, Zeh HJ, Whitcomb DC, Lyons-Weiler J. Feature Selection for Classification of SELDI-TOF-MS Proteomic Profiles.Appl Bioinformatics. 2005;4(4):227-46.

493. Numasaki M, Watanabe M, Suzuki T, Takahashi H, Nakamura A, McAllister F, Hishinuma T, Goto J, Lotze MT, Kolls JK, Sasaki H. IL-17 enhances the net angiogenic activity and in vivo growth of human non-small cell lung cancer in SCID mice through promoting CXCR-2-dependent angiogenesis. J Immunol. 2005 Nov 1;175(9):6177-89.

494. Lotfi R, Lotze M.T. Eosinophilic Granulocytes and Damage Associated Molecular Pattern Molecules [DAMPs]: Role in the Inflammatory Response Within Tumors. J Immunotherapy 2007 Jan;30(1):16-28.

495. Takahashi H, Numasaki M, Lotze MT, Sasaki H. Interleukin-17 enhances bFGF-, HGF- and VEGF-induced growth of vascular endothelial cells.Immunol Lett. 2005 May 15;98(2):189-93.

496. Tao R, Wang L, Chen CH, Wang SH, Demarco RA, Lotze MT, Thai NL, Fung JJ, Lu L, Qian S. Mechanistic insights into achievement of cardiac allograft long-term survival by treatment with immature DCs and sub-dose sirolimus. J Heart Lung Transplant. 2006 25:310-9.

497. Fujii S, Nishimura MI, Lotze MT. Regulatory balance between the immune response of tumor antigen-specific T-cell receptor gene-transduced CD8 T cells and the suppressive effects of tolerogenic dendritic cells. Cancer Sci. 2005 Dec;96(12):897-902.

498 D'Aiuto L, De Marco R, Edward N, Rizzo A, Chaillet JR, Montecalvo A, Lotze MT, Gambotto A. Evidence of the capability of the CMV enhancer to activate in trans gene DNA Cell Biol. 2006 Mar;25(3):171-80.

499. Eto M, Bennouna J, Hunter OC, Lotze MT, Amoscato AA. Importance of C16 ceramide accumulation during apoptosis in prostate cancer cells. Int J Urol. 2006 Feb;13(2):148-56.

500. Norimasa Ito, Richard A. DeMarco, Donna B. Stoltz, Michael T. Lotze. Identifying Type III (Necrotic) Cell Death At The Single Cell Level: Flow Cytometric Analysis of High Mobility Group B 1 and Histone H1. J Immunologic Methods, submitted.

501. Norimasa Ito, Richard A. Demarco, Robbie Mailliard, Jie Han, Hannah Rabinowich, Pawel Kalinski, Donna Stolz, Herbert J Zeh III, and Michael T. Lotze. Cytolytic Cells Induce HMGB1 Release from Melanoma Cell Lines. J Leukocyte Biology, 2007 Jan;81(1):75-83.

502. Izuishi K, Tsung A, Jeyabalan G, Critchlow ND, Li J, Tracey KJ, Demarco RA, Lotze MT, Fink MP, Geller DA, Billiar TR. Cutting edge: high-mobility group box 1 preconditioning protects against liver ischemia-reperfusion injury. J Immunol. 2006 Jun 15;176(12):7154-8.

503. Montag DT, Lotze MT. Rapid flow cytometric measurement of cytokine-induced phosphorylation pathways [CIPP] in human peripheral blood leukocytes. Clin Immunol. 2006 Nov;121(2):215-26.

504. Lotze MT, Yu Y. Cancer genomics: the unknown unknowns. Curr Opin Investig Drugs. 2006 Jun;7(6):497-500.

505. Nishioka Y, Wen H, Mitani K, Robbins PD, Lotze MT, Sone S, Tahara H. Differential effects of IL-12 on the generation of alloreactive CTL mediated by murine and human dendritic cells: a critical role for nitric oxide. J Leukoc Biol. 2003 May;73(5):621-9.

506. Vakkila J, DeMarco R, Lotze MT. Differential NF-κB and STAT1 activation in myeloid dendritic cells is dependent on their polarization. Submitted, J Immunology

507. Popovic PJ, Demarco R, Lotze MT, Winikoff SE, Bartlett DL, Krieg AM, Guo ZS, Brown CK, Tracey KJ, Zeh HJ 3rd. High Mobility Group B1 Protein Suppresses the Human Plasmacytoid Dendritic Cell Response to TLR9 Agonists. J Immunol. 2006 Dec 15;177(12):8701-7.

508. Wang Y, Zheng N, Lu Z, Wu W, Wang L, Nakao A, Lotze MT, Langer CE, Fung JJ, Qian S, Lu L. In vivo expansion of two distinct dendritic cells in mouse livers and its impact on liver immune regulation. Liver Transpl. 2006 Dec;12(12):1850-61.

509. Tsung A, Zheng N, Jeyabalan G, Izuishi K, Klune JR, Geller DA, Lotze MT, Lu L, Billiar TR. Increasing numbers of hepatic dendritic cells promote HMGB1-mediated ischemia-reperfusion injury. J Leukoc Biol. 2007 Jan;81(1):119-28.

510. Ito N, Zeh H, Lotze MT. Natural Killer Cells in *Encylopedia of Cancer*; Springer Verlag, Heidelberg, Germany 2007.

511. Lotze MT, Mapara M, June CH. Tumor Immunology and Immunotherapy for "Chapter 79. Clinical Immunology", 3rd Edition; Editors Rich, Shearer, Fleisher, Schroeder, Weyand, Frew;

Elsevier, London. 2007.

512. Rubartelli A, Lotze MT. Inside, outside, upside down: Damage associated molecular pattern molecules and Redox. Trends in Immunology, submitted [2007].

513. Ellerman JE, Brown CK, de Vera M, Zeh HJ, Billiar T, Rubartelli A, Lotze MT. Masquerader: high mobility group box-1 and cancer. Clin Cancer Res. 2007 May 15;13(10):2836-48.

514. Östberg T, Palmblad K, Wähämaa H, Ito N, Stridh-Igo P, Shoshan M, Lotze MT, Erlandsson Harris H and Andersson U. Oxaliplatin retains HMGB1 intranuclearly and transiently ameliorates collagen type II-induced arthritis in mice, submitted.

515. Zeh HJ, Winikoff S, Landsittel DP, Gorelik E, Marrangoni AM, Velikokhatnaya L, Winans MT, Lee K, Moser A, Bartlett D, Lotze MT, Siegfried JM, Whitcomb D, Slivka A, Bigbee WL, Lokshin AE. Multianalyte profiling of serum cytokines for detection of pancreatic cancer. Cancer Biomark. 2005;1(6):259-69.

516. Michael B. Atkins, David Carbone, George Coukos, Madhav Dhodapkar, Marc S. Ernstoff, James Finke, Thomas F. Gajewski, Jared Gollob, Michael T. Lotze, Walter Storkus and Louis M. Weiner Biologic Effects of Targeted Therapeutics, J Immunotherapy, in press 2007.

517. Lotze MT, Deisseroth A, Rubartelli A. FOCiS on damage-associated molecular pattern molecules. Clin Immunol. 2007 Apr 27; [Epub ahead of print]

ABSTRACTS AND PRESENTATIONS

1. Catalano, M.A., Carson, D.A., Lotze, M.T., Lotze, J., Slovin, S., and Vaughan J.H.: Antibodies to Epstein-Barr virus related nuclear antigens in two groups at different risks for rheumatoid arthritis. Clin. Res. 27:322A, 1979.
   Presented: American Assoc. Clin. Res., Washington, D.C. April 1979.

2. Lotze, M.T.: Human T cell cloning.
   Presented: Basel Inst. for Immun., Basel, Switzerland, Sept. 28, 1979.

3. Harvey, J., Lotze, M.T., Arnett, F.C., Bias, W.B., Billingsley, L.M., Harvey, E., Hsu, S., Sutton, J.D., Zizic, T.M., and Stevens, M.B.: Rheumatoid arthritis in a Chippewa population. Clin. Res. 28:147A, 1979.
   Presented: Southeastern Rheumatism Assoc., Greenbriar, West Virginia, Dec. 1, 1979.

4. Lotze, M.T.: Long term culture of human T cells.
   Presented: April 17, 1980; University of Rochester Cancer Center, Rochester, New York.

5. Lotze, M.T.: Cloning of human T cells.
   Presented: Norris Cotton Cancer Center, Dartmouth University, Hannover, New Hampshire, April 24, 1980.

6. Lotze, M.T.: Recent studies with the use of T cell growth factor.
   Presented: Research Institute of Scripps Clinic, La Jolla CA, May 27, 1980.

7. Harvey, J.H., Lotze, M.T., Bias, W.B., Hsu, S., Arnett, F.C., and Stevens, M.B.: Heterogeneity of DRw4 in a Chippewa population. Arth. & Rheum. 23:688, 1980.
   Presented: Amer. Rheum. Assoc., Atlanta, Georgia, May 27, 1980.

8. Lotze, M.T., Strausser, J., and Rosenberg, S.A.: Human T lymphocytes grown in T cell growth factor (TCGF): Cytotoxicity against autologous and allogeneic tumor. Proc. Amer. Assoc. Cancer Res. 21:216, 1980.
   Presented: Amer. Assoc. Cancer Res., San Diego, CA, May 31, 1980.

9. Duncan, M.A., Lotze, M.T., Gerber, L.H.,and Rosenberg,S.A.: Serratus anterior palsy following auxillary dissection: A prospective study. Arch. Phys. Rehab. 61:497, 1980.
   Presented: Amer. Acad. Phys. Med. & Rehab., Washington, D.C., Oct. 21, 1980.

10. Lotze, M.T., Lines, B.T., Strausser, J.L., and Rosenberg, S.A.: Tumor lysis by human T lymphocytes in long term culture and their distribution in vivo: Implications for immunotherapy.
    Presented: Amer. College of Surgeons, Atlanta, Georgia, October 21,1981.

11. Dubois, M., Lotze, M.T., Diamond, W.J., Kim, Y.D., Flye, M.W., and Macnamara, T.E.: Intraoperative acute respiratory failure induced by leucocyte antibodies. Crit. Care Med. 9:195, 1981.

Presented: World Cong. Intensive & Crit. Care Med.., Washington, D.C., May 24, 1981.

12. Lotze, M.T.: Research in progress: Lymphocyte clones.
Presented: Panel Discussion, Amer. Assoc. Histocompatability Testing, Orlando, Florida, May 24, 1981.

13. Lotze, M.T.: Surgical Perspectives; orders not to resuscitate.
Presented: Panel Discussion: Academy of Medicine, Rochester, New York, March 25, 1982.

14. Lotze, M.T.: An unusual radiologic manifestation of Buerger's Disease.
Presented: Hinshaw Day II; Rochester, New York, May 27, 1982.

15. Lotze, M.T., Marquis, D.M., Carey, A.S., Byer, B.J., Hoy, W.E., and May, A.M.: Two new assays for the early detection of transplant rejection, IL-2 response and PHA-augmented NK activity.
Presented: 3rd International Immunological Monitoring Symposium; Key Biscayne, Florida, November 22, 1982.

16. Lotze. M.T., Robb, R.J., Frana, L.W., Seipp, C.A., Sharrow, S.O., Chang, A.E., and Rosenberg, S.A.: Toxicity, Half life and immune effects of purified Jurkat derived interleukin-2 (IL-2) in patients with cancer and AIDS. J. Cell. Biochem. 8A:13, 1984.
Presented: 13th Annual UCLA Symposium: Acquired Immune Deficiency Syndrome; Park City, Utah, February 10, 1984.

17. Lotze, M.T., Robb, R.J., Frana, L.W., Seipp, C.A., Sharrow, S.O., Chang, A.E., and Rosenberg, S.A.:Systemic administration of Interleukin-2 in patients with cancer and AIDS: Initial results of a Phase I trial. Proc. Amer. Soc. Clin. Onc. 3:51, 1984.
Presented: American Society of Clinical Oncology; Toronto, Ontario.

18. Rayner, A.A., Grimm, E.A., Lotze, M.T., and Rosenberg, S.A.: Activated lymphocyte killing of fresh human tumor: Clonal analysis of a new approach to tumor immunotherapy.
Presented: Society of Surgical Oncology; New York, New York, May 14, 1984.

19. Rayner, A.A., Grimm, E.A., Lotze, M.T., and Rosenberg, S.A.: Demonstration of shared recognition and lysis of autologous and allogeneic fresh human tumors by cloned lymphokine activated killer cells (LAK): Implications for immunotherapy.
Presented: Assoc. for Academic Surgery; San Antonio, Texas, November 3,1984.

20. Lotze, M.T., Frana, L.W., Seipp, C.A., Sharrow, S.O., and Rosenberg,S.A.:Immunologic changes in patients with cancer given interleukin-2 (IL-2) in a Phase I trial.
Presented: Amer. Coll. Surgeons, San Francisco,CA; October, 1984.

21. Rayner, A.A., Grimm, E.A., Lotze, M.T., and Rosenberg, S.A.: Demonstration of shared

recognition and lysis of autologous and allogeneic fresh human tumors by cloned lymphokine activated killer (LAK) cells.
Presented: Amer. Coll. Surgeons, San Francisco, CA; October, 1984.

22. Lotze, M.T., and Rosenberg, S.A.: In vivo administration of interleukin-2 to humans.
Presented: 4th International Lymphokine Workshop, Klais, West Germany, October 20, 1984.

23. Lotze, M.T., and Rosenberg, S.A.: In vivo use of lymphokines.
Presented: Rochester Cancer Conference, September 28, 1984.

24. Lotze, M.T.: The Use of Human T cell Clones in Cellular Immunotherapy.
Presented: International Workshop on Human T-cell clones; Titisee, West Germany, March 7, 1985.

25. Lotze, M.T.: Current diagnosis and treatment of malignant melanoma.
Presented: St. John's Winter Seminar, Vail Colorado, March 7,1985.

26. Lotze, M.T.: Experience with the Administration of IL-2 to Humans.
Presented: Biogen, S.A. Geneva, Switzerland, February 26, 1985.

27. Lotze, M.T. and Rosenberg, S.A.: Current immunotherapy protocols using IL-2.
Presented: Beckton-Dickinson, Inc., Mountain View, CA May 29, 1985.

28. Lotze, M.T. and Rosenberg, S.A.: Clinical trials with recombinant IL-2.
Presented: Eastern Cooperative Oncology Group Meeting; Clearwater Beach, Florida; November 23, 1985.

29. Lotze, M.T.: IL-2 receptor expression on lymphocytes in patients treated with IL-2.
Presented: NIH Immunology Seminar, Bethesda, MD; December 3, 1985

30. Lotze, M.T.: IL-2 and LAK treatment of patients with cancer.
Presented: Norris Cotton Cancer Center; Dartmouth University; Hanover, NH; December 16, 1985.

31. Lotze, M.T.: Current treatment of hepatoma.
Presented: Georgetown University, Washington, D.C., March 15, 1985.

32. Rosenberg, S.A., Lotze, M.T., Muul, L.M., et al.: A new approach to the therapy of cancer based on the systemic administration of autologous lymphokine activated killer cells and recombinant interleukin-2.
Presented: Society of University Surgeons, Richmond, Virginia, February 3,1986.

33. Lotze, M.T.: LAK and IL-2 in the treatment of cancer.
Presented: Freie Universitat, Berlin, West Germany, March 4, 1986.

34. Lotze, M.T. and Rosenberg, S.A.: Current results with LAK and IL-2 in the treatment of cancer.
Presented: International Workshop on the Clinical Application of Lymphokines and Cytokines,Erlangen, West Germany, March 7, 1986.

35. Lotze, M.T. and Rosenberg, S.A.: Protocol design for phase I/II lymphokine studies in human cancer.
Presented: Meeting of the IUIS/WHO Lymphokine-Standardization Subcommittee, Erlangen, West Germany, March, 8, 1986.

36. Lotze, M.T.: Administration of LAK and IL-2 to patients with cancer.
Presented: St. Thomas' Hosp. Med. School; London, U.K., March 10, 1986.

37. Lotze, M.T.: IL-2 and LAK therapy.
Presented: D.C. Derm. Society Meeting, Bethesda, MD; March 25,1986

38. Lotze, M.T. and Rosenberg, S.A.: New approaches to the treatment of cancer: LAK and IL-2.
Presented: University of Rochester, Wasyl Pluta Cancer Center, April 16,1986.

39. Lotze, M.T. and Rosenberg, S.A.: IL-2 and LAK in the treatment of cancer.
Presented: St. Mary's Hospital, University of Rochester, April 16, 1986.

40. Roberts, K., Lotze, M.T. and Rosenberg, S.A.: Lymphokine activated killer tumor cell conjugates enumerated by flow cytometry. Fed. Proc.45:719, 1986.
Presented: 70th Annual Meeting, FASEB Meeting, St. Louis, MO; April 16, 1986.

41. Lotze, M.T.: Tumor Immunology.
Presented: FAES Course, IMM 522; Cell Biol. of Immun. and Inflamm. April 22, 1986.

42. Lotze, M.T., Carrasquilo, J.A., Weinstein, J.N., et al.: Monoclonal antibody imaging of human melanoma: Radioimmunodetection by subcutaneous or systemic injection.
Presented: 106th Meeting of the Amer. Surg. Assoc., Hot Springs, VA, April 24,1986.

43. Ognibene, F.P., Rosenberg, S.A., Skibber, J.M., Shelhammer, J.H., Lotze, M.T. and Parrillo, J.E.: Interleukin-2 hemodynamics mimic septic shock. Clin. Res. 34:413A, 1986.
Presented: American Federation Clinical Research, Washington, D.C., May 3, 1986.

44. Lotze, M.T. and Rosenberg, S.A.: Intraperitoneal administration of interleukin-2 in patients with cancer.
Presented: 39th Annual Cancer Symposium, Society of Surgical Oncology, Washington, D.C., May 12, 1986.

45. Lotze, M.T.: Possible clinical applications of Interleukin-2.
Presented: "Lymphokines", Breakfast Session, Society of Surgical Oncology,

Washington, D.C., May 14, 1986.

46. Ognibene, F.P., Rosenberg, S.A., Skibber, J.M., Shelhamer, J.H., Lotze, M.T. and Parrillo, J.E.: Interleukin-2 produces hemodynamic changes similar to septic shock in humans.
Presented: Society of Critical Care Medicine, Washington, D.C., May 27, 1986.

47. Lotze, M.T.: Current status of immunotherapy protocols.
Presented: Glenolden Labs, E.I.DuPont, Glenolden, PA, May 29, 1986.

48. Lotze, M.T.: Clinical applications of interleukin-2.
Presented: Grand Rounds, Mary Imogene Bassett Hospital, Cooperstown, NY, July 2, 1986.

49. Lotze, M.T. and Rosenberg, S.A.: Immunotherapy of cancer with high dose interleukin-2.
Presented: Sixth International Congress of Immunology, Toronto,Canada, July 7-8, 1986.

50. Lotze, M.T. and Rosenberg, S.A.: Immunotherapy of cancer with LAK cells and recombinant IL-2.
Presented: Chemotherapy of experimental and clinical cancer. Gordon Conference, New London, NH, July 20-25, 1986.

---

51. Lotze, M.T. and Rosenberg, S.A.: LAK cells and IL-2 in cancer therapy.
Presented: Congress on Research in Lymphokines and Other Cytokines, Basic Biology and Strategies for Clinical Application, Boston,MA, August 13, 1986.

52. Lotze, M.T. Clinical trials with IL-2 and monoclonal antibodies.
Presented: Oncogen, Seattle, Washington; August 21, 1986.

53. Lotze, M.T. Overview of LAK and IL-2 Therapy.
Presented: Ochsner Clinic; New Orleans, LA; October 20, 1986.

54. Lotze, M.T., Sugarbaker, P.H., Shawker, T.H. and Fortula, R.H.: Demonstration of intraoperative ultrasound imaging, CO_2 laser surgery and CUSA ultrasonic dissection for a right hepatic lobectomy for hepatoma.
Presented: Spectacular Problems in Surgery 72nd Annual Congress, American College of Surgeons, October 21, 1986.

55. Lotze, M.T., Biology of IL-2 and rationale for its clinical application.
Presented: In New Approaches to the Immunotherapy of Cancer; Conferences of the Combined Clinical Staff, NIH; October 28, 1986.

56. Lotze, M.T., Robert, K., Custer, M.C., Segal, D.A. and Rosenberg, S.A. Specific binding and lysis of human melanoma by IL-2 activated cells coated with anti-T3 or anti-Fc receptor crosslinked to anti melanoma antibody: A possible approach to the immunotherapy of cancer.

Presented: 20th Annual Meeting of the Association for Academic Surgery; Washington D.C., November 6, 1986.

57. Vetto, J.T., Papa, M.Z., Lotze, M.T., Chang, A.E. and Rosenberg, S.A. Reduction of toxicity of interleukin 2 and lymphokine activated killer cells in human by the administration of corticosteroids.
Presented: 20th Annual Meeting of the Association for Academic for Academic Surgery; Washington D.C., November 6, 1986.

58. Skibber, J.M., Lotze, M.T., Uppenkamp, I., Ross, W. and Rosenberg, S.A. Identification of human lymphokine activated killer cells: Implications for the immunotherapy of cancer.
Presented: Resident Research Award 20th Annual Meeting of the Association for Academic Surgery; Washington, D.C.; November 6, 1986.

59. Lotze, M.T. Treatment of hepatocellular carcinoma.
Presented: Hepatocellular Carcinoma; Conf. Combined Clinical Staff, NIH; Dec. 3, 1986.

60. Cohen, P., Lotze, M., Rosenberg, S., and Jaffee E.: The immunopathology of sequential tumor biopsies in patients treated with interleukin-2.
Presented: 76th Annual Meeting Int. Acad. Pathology; Chicago; March 9, 1987.

---

61. Lotze, M.T. Clinical use of interleukin-2.
Presented: Joint Mtg. French and Swiss Soc. of Immunology; Geneva, April 23, 1986.

62. Tomita, S., Lotze, M.T. and Rosenberg, S.A.: Clonal analysis of tumor infiltrating lymphocytes against human malignant melanoma. Fed. Proc. 46:1195, 1987.
Presented: 71st Annual Meeting FASEB; Washington, D.C. March 1, 1987.

63. Wiebke, E.A., Lotze, M.T. and Rosenberg, S.A.:Human tumor susceptibility to lymphokine activated killer (LAK) lysis: interferon and tumor necrosis factor decrease NK but not LAK lysis. Fed. Proc. 46:1510, 1987.
Presented: 71st Annual Meeting FASEB; Washington, D.C.; March 2, 1987.

64. Lotze, M.T. Adoptive Immunotherapy of Cancer.
Presented:4th Annual Advances in Cancer Treatment Research Symposium; New York, March 27, 1987.

65. Lotze, M.T. Current status of NIH trials using IL-2.
Presented: Rec. Adv. in Cancer Ther.; University of Colorado; Denver, March 10, 1987.

66. Lotze, M.T.: New approaches to the immunotherapy of cancer.
Presented: American Cancer Society; Rockville, MD, March 7, 1987.

67. Lotze, M.T.: Adoptive immunotherapy.

Presented: Keynote Address: 2nd International Head & Neck Oncology Research Conference. Arlington, Virginia; September 10-12, 1987.

68. Garra, B.S., Insana, M.F., Fisher, B., Lotze, M.T., Shawker, T.H., Wagner, R.F., Russell, M.A.: Quantitative ultrasonic detection of liver changes in patients undergoing interleukin-2 therapy.
Presented: 13th International Symposium on Ultrasound Imaging and Tissue Characterization. Arlington, VA; June 7, 1988.

69. Lotze, M.T.: Disturbing homeostasis: New approaches to the immunotherapy of cancer.
Presented: Distinguished Visting Professor; Uniformed Services, University for the Health Sciences, Bethesda, MD; January 28, 1988.

70. Lotze, M.T.: 1) Therapy with monoclonal antibodies and IL-2; and, 2) Clinical use of lymphokines.
Presented: Bristol Myers Spring Scientific Symposium, Wallingford, CN; March 3, 1988.

71. Lotze, M.T.: Disturbing hemostasis: New approaches to the immunotherapy of cancer.
Presented: Immunotherapeutics; Spring Branch Memorial Hospital, Houston, TX; March 19, 1988.

72. Lotze, M.T.: New approaches to the immunotherapy of cancer.
Presented: Yale University, March 22, 1988.

73. Lotze, M.T.: Immunotherapy of cancer.
Presented: Cancer Center Grand Rounds Howard University, Washington, DC; March 31, 1988.

74. Lotze, M.T.: Preclinical studies with Interleukin-4.
Presented:Eastman Pharmaceuticals, Great Valley,PA April 12, 1988.

75. Lotze, M.T.: Disturbing homeostasis: recent results of ongoing immunotherapy trials at the NCI.
Presented: Recent Updates in Basic Research and Clinical Development of Biological Response Modifiers, Chicago, IL; April 13, 1988.

76. Lotze, M.T.: Disturbing homeostasis: Current status of NCI immunotherapy trials.
Presented: Interleukin-2: Clinical and Biological Update, Roswell Park Memorial Institute, Buffalo, NY; April 20, 1988.

77. Lotze, M.T.: Human tumor antigens defined by cytotoxic and proliferative T cells. J. Cell Biochem 12E:121, 1988.
Presented: Cetus-Triton Biosciences UCLA Symposium Human Tumor Antigens and Specific Tumor Therapy, Keystone, CO; April 26, 1988

78. Kawakami, Y., Custer, M., Rosenberg, S.A., and Lotze, M.T.: Human interleukin-4 (IL-4)

inhibits Interleukin-2 (IL-2) induction of human lymphokine activated killer (LAK) activity from peripheral blood and spleen cells. FASEB JI2:A660, 1988.
Presented: FASEB Meeting; Las Vegas, Nevada; May 2, 1988.

79. Jablons, D., Keenan, A., Yolles, P.S., Fisher, B., and Lotze, M.T.: The use of computerized Tech-99 DISIDA hepatobiliary scanning to evaluate liver function in the preoperative evaluation of patient's undergoing liver resection.
Presented: 41st meeting of the Soc. of Surg. Oncology, New Orleans, LA.

80. Lotze, M.T.: Immunotherapy of melanoma.
Presented: Melanoma Update, St. Louis Community Oncology Program, St. Louis, MO; June 10, 1988.

81. Sakahara, H., Carrasquillo, J.A., Lotze, M.T., Reynolds, J.C., Bryant, G., Perentesis, P., Lerario, L., Green, S., and Larson, S.M.: Effect of Interleukin-2 on biodistribution of I-131 labeled monoclonal antibody 9.2.27. J. Nucl. Med. 29:897, 1988.
Presented:Society of Nuclear Medicine, San Francisco, CA; June 1988.

82. Sakahara, H., Reynolds, J.C., Carrasquillo, J.A., Lora, M., Lotze, M.T., and Larson, S.M.: *In vitro* complex formation and serum clearance of mouse monoclonal antibody. J. Nucl. Med. 29:758, 1988.
Presented:Society of Nuclear Medicine, San Francisco, CA; June 1988;

83. Huang, C,M., Ruddel, M., Sliva, C., Elin, R.J., Lotze, M.T., and Rosenberg, S.A.: The effect of Interleukin-2 (IL-2) on tests of liver function.
Presented: American Association of Clinical Chemistry

84. Lotze, M.T.: Immunotherapy of cancer.
Presented: 14th SVU World Cong., Chevy Chase, MD; September 15, 1988.

85. Lotze, M.T.: Use of lymphokines in therapy.
Presented: XIII International Congress of Allerology and Clinical Immunology, Montreux, Switzerland; October 21, 1988.

86. Lotze, M.T.: Clinical application of lymphokines.
Presented: VIth International Lymphokine Workshop, Evian, France; October 25, 1988..

87. Kawakami, Y., Rosenberg, S.A., and Lotze, M.T.: Interleukin-4 (IL-4) promotes the growth of TIL specific for human autologous melanoma.
Presented: 6th International Lymphokine Workshop, Evian, France; October 25, 1988.

88. Kawakami, Y., Rosenberg, S.A., and Lotze, M.T.: Interleukin-4 (IL-4) promotes the growth of tumor infiltrating lymphocytes (TIL) specific for human autologous melanoma.
Presented: 6th International Lymphokine Workshop, Evian, France; October 26, 1988.

89. Lotze, M.T.: Current Status of Immunotherapy Trials by the Surgery Branch of NCI.
Presented: President's Cancer Panel; Chair, Dr. Armand Hammer; November 7, 1988.

90. Jablons, D.J., McIntosh, J.D., Mute, J.J., Nordan, R.P., Rudikoff, and Lotze, M.T.: Induction of Interferon B_2/interleukin-6 (IL-6) by cytokine administration and detection of circulation interleukin-6 in the tumor bearing host.
Presented: Regulation of the Acute Phase and Immune Responses: A new Cytokine; New York Academy of Sciences, NY; December 12, 1988.

91. Lotze, M.T.: IL-4 regulates responsiveness to IL-2.
Presented:NIH-Wide Immunology Seminar Series, Bethesda, MD. December 13, 1988.

92. Lotze, M.T.: T Cell reactivity against human cancer.
Presented: Hammer Symposium; LaJolla, CA, January 5, 1989.

93. Lotze, M.T.: Interleukin-2 /LAK cell therapy.
Presented: Oncology Viewpoints, Mod. W.L. McGuire, San Antonio, TX, Jan. 10, 1989.

94. Lotze, M.T.: LAK cells.
Presented: Student Immunology Lecture Series; Bethesda, MD; February 8, 1989.

95. Lotze, M.T.: Cardiac and hemodynamic effects of IL-2.
Presented: Gerontology Research Center, NIA; Baltimore, MD, February 9, 1989.

96. Cornetta, K., Culver, K., Morecki, S., Kasid, A., Morgan, R., Aebersold, P., Lotze, M.T., Rosenberg, S., Anderson, W.F. and Blaese, R.M.: Retroviral vectors and Human Tumor infiltrating lymphocytes: In vitro findings.
Presented: 30th Annual Meeting, American Society of Hematology.

97. Rosenberg, S.A., Lotze, M.T., Yang, J., Chang, A.E., Seipp, C., Simpson, Results of treatment of 650 patients with interleukin-2.
Presented: 109th Annual Meeting, American Surgical Assoc.

98. Lotze, M.T.: "Studies of T Cell Growth Factors: New Approaches Using IL-2, IL-4 and IL-6".
Presented: University Wisconsin Oncology Grand Rounds, Madison Wisconsin, January 25, 1989.

99. Jablons, D., Bolton, Mertons, S., Rubin, M., Pizzo, P., and Lotze, M.T.: Interleukin-2 (IL-2) administration to cancer patients alters neutrophil FcR expression, superoxide response and chemotaxis.
Presented: 73rd Annual Meeting, FASEB, New Orleans; March 23, 1989.

100. Lotze, M.T.: Immunotherapy of cancer.

Presented: Memorial-Sloan Kettering Institute, NY. May 19, 1989.

101. Lotze, M.T., and Balch, C.: Cochairs, T cells and Tumor infiltrating lymphocytes, Minisymposium.
Presented: American Assoc. Cancer Research Annual Meeting, San Francisco, May 25, 1989.

102. Lotze, M.T.: Toxicity of IL-2 treatment.
Presented: Safety Assessment of Cytokines, USUHS, Bethesda, MD, May 23, 1989.

103. Lotze, M.T.: Biologic therapy: An Effective Fourth Modality of Cancer Treatment.
Presented: 75th Anniversary, Daniel Van Hoed Cancer Radiobiologic Institute; Rotterdam, The Netherlands, June 2, 1989.

104. Lotze, M.T.: Biologic Therapy of Cancer
Presented: Ohio State University, Columbus Ohio, June 5, 1989.

105. Lotze, M.T.: Preclinical and clinical application of IL-4.
Presented: Therapeutic Applications Meeting Biologic Resp. Mod. Program; Frederick MD, June 15, 1989.

106. Lotze, M.T.: Cytokine therapy of patients with cancer.
Presented: Plenary Session, Immune Intervention; 7th International Congress of Immunology Berlin; August 1, 1989.

107. Kawakami, Y., Lotze, M.T.: Interleukin-4 promotes growth of human tumor infiltrating lymphocytes.
Presented: 7th International Congress of Immunology, Berlin; August 4, 1989.

108. Lotze, M.T.: Mechanisms of Immunologic Antitumor therapy: Lessons from the laboratory and clinical applications.
Presented: 2nd International Conference on Cells Invading the Rejecting allograft; Pittsburgh, September 15, 1989.

109. Lotze, M.T.: Immunologic effects of IL-4 in man
Presented: NIH Immunology noon seminar; October 24, 1989.

110. Rubin, J., Lotze, M.T.: Interleukin-2 and the adoptive therapy of cancer.
Presented: Int. Interleukin-2 Symposia. Manchester, United Kingdom; October 25, 1989.

111. Lotze, M.T.: Cytokines and Cancer Therapy.
Presented: Williamsburg Immunology Conference, Williamsburg, November 18, 1989.

112. Lotze, M.T.: Biologic Therapy: An effective fourth modality of cancer treatment.

Presented: Surgical Grand Rounds, Duke University, November 29, 1989.

113. Lotze, M.T.: T cells and the treatment of cancer patients.
Presented: Surgical Grand Rounds, University of Utah, Salt Lake City, Utah; January 31, 1990.

114. Lotze, M.T.: Fundamentals of cancer ontogeny and immunotherapy.
Presented: Graduate Seminar, University of Utah,Salt Lake City, Utah; February 1, 1990.

115. Lotze, M.T.: Use of recombinant human IL-2 and IL-4 in vitro and in vivo to expand TIL's. J. Cellular Biochem, 14B:61, 1990.
Presented: UCLA Symposium on Cellular Immunity; Park City, Utah; and the immunotherapy of Cancer; February 1, 1990.

116. Jablons, D.H., Lotze, M.T.: Enhanced expansion of cells with lymphokine activated killer cell (LAK) activity from human b one marrow and peripheral blood - implications for immunotherapy. J. Cellular Biochem 14:72, 1990.
Presented: UCLA Symposium on Cellular Immunity and the immunotherapy of Cancer; Park City, Utah; February 1, 1990.

117. Lotze, M.T.: Progress in immunotherapy.
Presented: ACS - UVA Conference on cancer; Charlottesville, VA, February 16, 1990.

118. Lotze, M.T.: Application of Biologic therapy to patients with cancer.
Presented: Surgical Grand Rounds, University of Virginia; Charlottesville, VA; February 16, 1990.

119. Lotze, M.T.: Application of cytokines to the treatment of cancer.
Presented: Advances in cancer diagnosis and therapy; Fort Lauderdale FL; March 1, 1990.

120. Lotze, M.T.: Adoptive immunotherapy of cancer.
Presented: 4th Annual advances in cancer treatment research (Montefiore/Albert Einstein); New York, NY: March 8, 1990.

121. Lotze, M.T.: Use of cytokines in cancer treatment.
Presented: First International Cytokine Congress; Florence, Italy; March 26, 1990.

122. Lotze, M.T.: Biologic therapy - an effective form of cancer treatment.
Presented: 14th Annual lectureship, Internal Medicine Group; New Orleans, LA; March 29, 1990.

123. Lotze, M.T.: Advances in cancer immunotherapy.
Presented: Cancer Progress V/ Communitech Market Intelligence Inc; New York, NY;

April 23, 1990.

124. Lotze, M.T.: Tumor Immunology
Presented: FAES/Immunology 502; Cell Biology of Immunity and Inflammation; Bethesda, MD; May 1, 1990.

125. Lotze, M.T.: Restricted T cell receptor usage in recognition of human melanoma.
Presented: Dept. of Molecular Genetics and Biochemistry, University of Pittsburgh; Pittsburgh, PA; May 7, 1990.

126. Bolton, E., Custer, M. and Lotze, M.T.: Interleukin-4 (IL-4) alters monocyte phenotype in vitro and in vivo.
Presented: Amer. Assoc. Cancer Res.; Washington, D.C.; May 31, 1990.

127. Stotter, H., Haas, H., Lotze, M.T. and Rosenberg, S.A.: Pretreatment of renal cell cancer (RCC) patients with alpha interferon (IFNá) and Interleukin-2 (IL-2) prior to nephrectomy.
Presented: ASCO, Washington, D.C., May 22, 1990.

128. Lotze, M.T.: Biologic Response Modifiers - Clinical Trial Research.
Presented: Colorectal Cancer Symp./Brown University, Providence, RI; June 2, 1990.

---

129. Kawakami, Y., Kumar, V., Hood, L., Rosenberg, S.A. and Lotze, M.T.: Unique TCR rearrangements in melanoma TIL.
Presented: Amer. Assoc. Immunology Mtg; New Orleans, LA, May 6, 1990.

130. Lotze, M.T.: Adoptive Immunotherapy of Cancer.
Presented: Amer. Assoc. Immunology/Plenary Session; New Orleans, LA: May 6, 1990.

131. Lotze, M.T.: T-Time: Use of T-cell growth factors to treat patients with cancer.
Presented: Clinical Center Grand Rounds/NIH; Bethesda, MD; August 1, 1990.

132. Lotze, M.T.: Treatment of HCC (moderator) and immunological modalities of treatment; Radiological diagnosis of HCC (with Irwin Feuerstein).
Presented: Hepatocellular carcinoma in North America; Bethesda, MD; Sept. 26-27, 1990.

133. Choyke, P. L., Miller, D.L, Lotze, M.T., Whites, J. M., Ebbit B. Delayed reactions with non-ionic contrast media in association with IL-2 treatment.
Presented: 76th Scientific Assembly of the Radiol. Soc. of North Amer.; Nov. 25, 1990.

134. Lotze, M.T.: Advances in cancer immunotherapy.
Presented: Amer. Coll. Surgeons Course, San Francisco, CA; October 12, 1990

135. Lotze, M.T.: Adoptive Immunotherapy - Current Status.

Presented: Clin. Immunology Society, Chicago, Illinois; November 9, 1990.

136. Lotze, M.T.: Treatment of metastatic colorectal cancer.
Presented: Annual Pittsburgh Cancer Conference; December 7, 1990

137. Zeh, H.J. III, Lotze M.T., Wang S. Detection of cytokine mRNA in mitogen activated peripheral blood lymphocytes by RT-PCR. J Immunother 11:152, 1992.
Presented: Society of Biologic Therapy, Pittsburgh, PA; November, 1991.

138. Storkus W.J., Lotze M.T. Melanoma Immunogenicity: Melanoma Cells Present Both Endogenously - and Exogenously - Derived Peptides to CD8+ cytolytic T-cells. J Immunother 11:147, 1992
Presented: Society of Biologic Therapy, Pittsburgh, PA; November, 1991.

139. Rubin J.T., Adams S., Simonis T., Lotze M.T. HLA - Polymorphism and Response to IL-2 - Based Therapy in Patients with Melanoma.J Immunother 11:141-142, 1992.
Presented: Society of Biologic Therapy, Pittsburgh, PA; November, 1991.

140. Cai Q, Samulski R.J., Ricordi C, Lotze MT. Adeno-associated Virus (AAV) Can be Used as a Potential Vector to Transfer Genes into Pancreatic Islets. J Immunother 11:122-123, 1992.
Presented: Society of Biologic Therapy, Pittsburgh, PA; November, 1991.

141. Pippin B, Cai Q, Lotze M.T. Evaluation of Immune Reactivity to IL-2 Transfected Tumors. J Immunother 11:137-138, 1992.
Presented: Society of Biologic Therapy, Pittsburgh, PA; November, 1991.

142. Pockaj B.A., Lotze M.T., Yang J, Steinberg S, Rosenberg S.A. A Prospective Randomized Trial Evaluating Crystalloid versus Colloid Fluid Resuscitation for Interleukin-2 Based Therapy. J Immunother 11:138-139, 1992.
Presented: Society of Biologic Therapy, Pittsburgh, PA; November, 1991

143. Leder G.H., Finley G.C., Rubin J.T., Pipas J.M., Law J, Lotze M.T. Mutant p53 as a Target for Immune Recognition. J Immunother 11:131-132, 1992.
Presented: Society of Biologic Therapy, Pittsburgh, PA; November, 1991

144. Elder E.M., Kuebbing D., Lotze M., Whiteside T.L. Selection of Neomycin-Resistant TIL Obtained from Human Melanoma and Cultured in the Presence of IL-2 and IL-4. J Immunother. 11:125, 1992.
Presented: Society of Biologic Therapy, Pittsburgh, PA; November, 1991

145. Tahara, H. Zeh III, HJ, Mueller Gm, Gately MK, Gubler U, Wolf S, Robbins PD and Lotze MT. Cancer Vaccination using Interleukin-12 (IL-12) Gene Transfer.
Presented: Cold Spring Harbor Laboratory. September 22, 1992.

146. Storkus, WJ, Hauser, T, Lotze, MT, and Dawson JR. The role of peptide-self in class I-mediated NK resistance.
Presented: NK Workshop, Ft. Lauderdale, Fl., October 5, 1992.

147. Pippin B.A., Kuebbing D., Nishihara K., Hurd S.D., Lotze M.T. Transfection of Interleukin-4 into fibroblast for cytokine gene therapy of cancer. J Immunother 13:69, 1993.
Presented: Society of Biologic Therapy, Williamsburg, VA; Nov. 1992.

148. Berman R.M., Zeh H.J., Storkus W.J., Lotze M.T. Interleukin-10 Induces Lymphokine Activated Killer (LAK) Cell Activity. J Immunotherapy 13:56, 1993.
Presented: Society of Biologic Therapy, Williamsburg, VA; Nov. 1992.

149. Oppenheim M., Rao P., Lotze M.T. Serum Hyaluronan Levels Increase with Interleukin-2 Therapy. J Immunotherapy 13:68,1993.
Presented: Society of Biologic Therapy, Williamsburg, VA; Nov. 1992.

150. Lotze MT. T-Cell Growth Factors and the Treatment of Cancer.
Presented: Cancer Ctr Grand Rounds, Univ. of Michigan, Ann Arbor, MI, Jan. 22, 1993.

151. Lotze M.T. IL-2 and IL-12 in the Treatment of Cancer.
Presented: Cetus/Chiron Corp.; Emeryville, CA; January 28, 1993.

152. Lotze MT and Herberman RB. 1) Overview of cytokine treatment: IL-2 to IL-12 2) Adoptive Immunotherapy of Cancer.
Presented: Molecules to Medicine Symposium. Second international Congress on Biol. Response Modifiers. San Diego CA, January 29-31, 1993.

153. Lotze MT. 1) New approaches to cytokine therapy; 2) Current status of adoptive immunotherapy.
Presented: Biologic Response Modifiers Steering Committee/Eastern Cooperative Oncology Group Meeting; Atlanta, GA. February 10-11, 1993.

154. Tahara H, Zeh H, Pappo I, Nastala C, Robbins PD, Lotze MT. Tumor growth alteration with local Interleukin-12 secretion achieved by gene transfer.
Presented: Society of University Surgeons; Montreal, Quebec; February 11-13, 1993.

155. Lotze MT. Workshop on Antagonists of Cytokine Function.
Presented: Keystone Symposium On "Cytokines and Cytokine Receptors: From Cloning to the Clinic"; Keystone, CO; February 6, 1993.

156. Lotze MT. Transfer of Gene Marked TILs; Current Status and Future Goals.
Presented: International Congress on "Biological Response Modifiers: Present Clinical Use and Future Developments"; Naples, Italy; February 23, 1993.

157. Lotze M.T. Gene Therapy of Cancer - Immunological Approaches.
Presented: NCI Gene Therapy Working Group; Rockville, MD; March 1, 1993.

158. Storkus W.J. and Lotze M.T. Identification of Human Melanoma derived Epitopes Recognized by HLA-A2 Restricted , CD8+ Tumor Infiltrating Lymphocytes. J. Cell Biochem. 17D: ,1993.
Presented: Keystone Symposium on "Cellular Immunity and Immunotherapy of Cancer"; Taos, NM; March 19, 1993.

159. Lotze M.T. Modulation of Murine Reactivity to Tumor and Transplantation Antigens. J. Cell. Biochem.17D: ,1993.
Presented: Keystone Symposium on "Cellular Immunity and Immunotherapy of Cancer"; Taos, NM; March 19, 1993.

160. Lotze M.T. and Whiteside T.L. Immunology and Biological Therapeutics.
Presented: PCI Scientific Retreat; Nemacolin, PA; March 28, 1993.

161. Lotze M.T. WJ Storkus, SD Hurd, MJ Maeurer, JM Kirkwood. Immunotherapy of Melanoma. Melanoma Res. 3:5-6, 1993.
Presented: Third International Conference on Melanoma; Special Lecture; Venice, Italy; April 3, 1993.

---

162. Lotze M.T., Cai Q., Elder E., Rubin J, Pippin B., Jacob W., Chen Y., Nishihara K., Siegfried J., Storkus W., Edington H, Rosenstein M, Nastala C, Pappo I, Zitvogel L., Robbins P., Tahara H. Gene Therapy of Cancer - Immunological Approaches. J. Cell. Biochem. 17E:184, 1993.
Presented: Keystone Symposium on "Genetically Targeted Research and Therapeutics: Antisense and Gene Therapy"; Keystone, CO; April 12, 1993.

163. Ragni M.V., Lotze M.T. Hemophilia: An Important Target for Gene Therapy. J. Cell. Biochem. 17E:216,1993.
Presented: Keystone Symposium on "Genetically Targeted Research and Therapeutics:Antisense and Gene Therapy"; Keystone, CO; April 12, 1993.

164. Tahara J., Zeh H. III, Pappo I., Nastala C., Robbins P.D., Lotze M.T. Tumor Growth Alteration with Local Interleukin 12 Secretion Achieved by Gene Transfer. J Cell Biochem. 17E. 247, 1993.
Presented: Keystone Symposium on "Gene Therapy"; Keystone, CO; April 12, 1993.

165. Lotze M.T. Biotherapy of Cancer.
Presented: CILAC XI; 11th Congresos Integrados Latino Americanos De Cancerologia; Cancun, Mexico; May 12, 1993.

166. Lotze MT and Levy R.: New approaches to immunotherapy and vaccines. against cancer. J Immunol, 150: 1A, 1993.
Presented: Joint Meeting of AAI and CIS, Denver, CO; May 22, 1993.

167. Lotze M.T.: A Tale of Two Cytokines: IL-4 and IL-12.
Presented: Multidisciplinary Program in Immunology/Stanford University Medical Center; Stanford, CA; June 2, 1993.

168. Lotze M.T.: Cytokine Gene Therapy of Cancer.
Presented: Monthly Lecture Series, New England Deaconess Hospital/Department of Surgery; Boston, MA; June 10, 1993.

169. Lotze MT: Eluted peptides from the MHC.
Presented: First International Conference on Engineered Cancer Vaccines and AIDS; San Francisco, CA; Sept. 30- October 3, 1994.

170. Pappo I, Tahara H, Nastala C, Robbins PD, Zeh HJ, Lotze MT. Cancer gene therapy with IL-12 alone or in combination with systemic IL-2 administration delays or prevents the growth of murine sarcomas.
Presented: Assoc. Academic Surgery. Hershey, PA; Nov. 13, 1993.

171. Pappo I, Wasserman K, Tahara H, Epperly MW, Bryant J, Lotze MT, Rosenstein MM. The systemic administration or local delivery of IL-12 combined with radiation (RT) delays the growth of a virulent murine melanoma.
Presented: Association Academic Surgery. Hershey, PA; November 12, 1993.

172. Nastala C, Edington H, Storkus WJ, Lotze MT. Recombinant Interleukin-12 (rmIL-12) Mediates Regression of Both Subcutaneous and Metastatic Murine Tumors.
Presented: 79th Annual Clinical Congress; American College of Surgeons. San Francisco, CA; October 10, 1993.

173. Leder GH, Oppenheim M, Rosenstein M, Shah N, Hoffman R, Simmons R, Lotze MT. Aminoguanidine decreases IL-2 induced nitric oxide production but not the IL-2 induced capillary leak syndrome.
Presented: 3rd Int. Mtg; Biology of Nitric Oxide. Cologne, Germany; October 3, 1993.

174. Lotze MT. Interleukin 4 and Interleukin 12: Cytokines that regulate the immune response. Annals of Hematology 67:A173,S4, 1993.
Presented: Advances in Cytokine Development, Munich, Germany; October 27, 1993.

175. Lotze MT. Interleukin 4 Gene Therapy. Annals of Hematology 67:A171,111, 1993.
Presented: Cytokines and Growth Factors in Cancer: From Basic Research to Clinical Application. Munich, Germany, October 30, 1993.

176. Lotze MT. A Tale of Two Cytokines - IL-4 and IL-12 Regulate Immune Reactivity.
Presented: US-Japan Cancer Cooperative Research Program, "Cell Biology of the Host Antitumor Immune Response". Rockville, MD January 10-12, 1994.

177. Lotze MT. IL4 and IL12 Gene Therapy: Cytokines which Regulate the Immune Response.
Presented: First International Conference on Gene Therapy and Vaccines for Cancer. Washington, DC January 27, 1994.

178. Lotze MT. Gene Altered TIL: Background and Rationale.
Presented: Third International Symposium on The Biology of Renal Cell Carcinoma. Cleveland, OH March 8, 1994.

179. Leder G, Oppenheim M, Rosenstein M, Hoffman R, Lotze M, Beger H. NO does not mediate IL-2 induced antitumor effects. Br. J. Surg 81:102, 1994.
Presented: European Surgical Society, Fall 1994.

180. Smith DC, Jacob HE, Lotze MT, Branch RA, Adedoyin A, Stiff D, Ellis PG, Schwartz K, Trump DL. A phase I trial of interferon-α2a (IFN-α) and all-transretinoic acid (ATRA): A pharmacokinetic assessment. Proc. ASCO 13:134 (#329), 1994
Presented: American Society of Clinical Oncology, May 1994.

181. Lotze MT. Gene Altered TIL: Background and Rationale.
Presented: Third International Symposium on The Biology of Renal Cell Carcinoma. Cleveland, OH March 8, 1994.

182 Lotze MT. Cytokine therapy of Cancer - IL-4 and IL-12 Regulate the Immune Response. Brit. J. Cancer 69:Supplement XXI, 2(S6), 1994.
Presented: BACR/ACP Annual Meeting. Birmingham, United Kingdom; March 28, 1994.

183. Maeurer M, Castelli C., Hurd S., Martin D., Storkus W., Lotze M. In vivo selection of melanoma variants lacking CTL-defined epitopes. FASEB Jl 8:A209 (1203), 1994.
Presented: Experimental Biology 94 (American Assoc. of Immunology); Anaheim, CA; April 24, 1994.

184. Qin L, Chavin KD, Tahara H, Robbins PD, Lotze MT, Bromberg JS. IL-10 gene transfer prlongs cardiac allograft survival. FASEB Jl. 8:A738 (4285), 1994.
Presented: Experimental Biology 94 (American Assoc. of Immunology); Anaheim, CA; April 26, 1994.

185. Vokes E, Hochster H, Lotze M, Figlin R, Rybak ME. Recombinant human interleukin 4 (rhu IL-4) SCH 39400 in non-small cell lung cancer (NSCLC): preliminary results of a phase II investigation. Proc. ASCO 13:334 (#1107), 1994.
Presented: American Society of Clinical Oncology, Dallas, TX; May 1994.

186. Lotze MT. Interleukin 4 and IL-12 regulate immune responses.
Presented: Immunology Seminar, Pittsburgh Cancer Institute; May 1994.

187. Lotze MT. Role of Biologic Agents in Treating Pancreatic Cancer.
Presented: Arthur W. Beauregard International Cancer Conference. Cancer of the Pancreas: Challenge of the Nineties. Newport, Rhode Island; July 5-8, 1994.

188. Zitvogel L, Tahara H, Storkus WJ, Robbins P, Lotze MT. IL-12 Gene Therapy.
Presented: J.P. Lecocq Conference on Gene Therapy, Strasbourg, France; July 5-7, 1994.

189. Suminami Y, Elder EM, Lotze MT, Whiteside TL. Quantitative PCR for expression of the IL4 gene in biopsies of patients receiving genetically modified tumor vaccine.
Presented: Society of Biological Therapy Meeting, Silverado, CA; October 26-30, 1994.

190. Mayordomo JI, Storkus WJ, Deleo R, Lotze MT, DeLeo AB. A CTL clone specific for the Meth A murine sarcoma successfully treats established metastatic disease.
Presented: Society of Biological Therapy Meeting, Silverado, CA; October 26-30, 1994.

191. Zorina T, Mayordomo JI, Watkins S, Lotze MT, DeLeo AB, Ildstad ST. Culture of dendritic cells from murine bone marrow supplemented with GM-CSF and TNF-alpha.
Presented: Society of Biological Therapy Meeting, Silverado, CA; October 26-30, 1994.

192. Posner MC, Lembersky B, Landreneau RJ, Mullen E, Oppenheim M, Lotze MT. Combined modality therapy for operable carcinoma of the esophagus and gastroesophageal (GE) junction. Proc. ASCO 12:224 (686), 1993
Presented: American Society of Clinical Oncology, Orlando, May 1993.

193. Lotze MT. T-cell Factors in Cancer Immunotherapy.
Presented: Second International Cytokine Conference. Banff, Alberta October 5, 1994.

194. Berman R, Suzuki T, Tahara H, Robbins P, Lotze M. Human and viral Interleukin-10 (cIL-10 and vIL-10) mediate opposing effects in tumor immunity.
Presented: Second International Cytokine Conference. Banff, Alberta October 4, 1994.

195. Lotze MT, Tahara H, Storkus WJ, Zitvogel L, Suzuki T, Berman R, Robbins PD. The non-γ_cR T-cell growth factors - cytokine gene therapy for cancer and transplantation. Gene Therapy 1:S4(A12), 1994.
Presented: Second Meeting of the European Working Group on Human Gene Transfer and Therapy. London, UK. November 19, 1994.

196. Lotze MT. Cytokine and Cytokine Gene Therapy of Cancer.
Presented: Seventh Meeting of the Japanese Society of Biologic Response Modifiers. Tokushima, Japan. December 2-3, 1994.

197. Lotze MT. The Immune System Connection to Cancer and Transplantation.
Presented: Cancer Research and Treatment; Beyond the Year 2000: Harnessing the

Immune System. Sponsored by the Cancer Research Institute and Immunex; January 12, 1995; New York City, New York.

198. Lotze Michael T. Cytokines and Vaccines For Tumor Treatment.
Presented: Committee on Immunology, University of Chicago, January 16,1995; Chicago, IL.

199. Lotze MT. Melanoma: From the clinic to the laboratory and back again.
Presented: Pittsburgh Surgical Society, January 23, 1995.

200. Lotze MT. Cytokines for Cancer Therapy.
Presented: International Biologic Response Modifier Symposium; January 27, 1995, Cancun, Mexico

201. Lotze Michael T. Discussant.
Ciba Foundation Symposium No. 195. T Cell Subsets in Infectious And Autoimmune Diseases, March 6-10, 1995, London, UK.

202. Rubin JT, Brumfield A, Dookeran K, Lotze MT. Tumor targetted delivery of sustained release high-dose 9-aminocamptothecin. Proc. AACR 36:452(2695), 1995
Presented: 86th Annual Meeting of the American Association of Cancer Research, March 21, 1995; Toronto, Canada.

203. Mayordomo J, Frassanito AM, DeLeo RM, Storkus WJ, Lotze MT, DeLeo AB. Development of CTL-defined tumor peptide vaccine models using chemically induced BALB/c sarcomas. Proc. AACR 36:493(2936), 1995.
Presented: 86th Annual Meeting of the American Association of Cancer Research, March 21, 1995; Toronto, Canada.

204. Lotze MT. Tumor Immunology - The New Biology.
Presented: American Association for the Advancement of Science; February 21, 1995; Atlanta, GA.

205. Lotze MT. Effective Cytokine Gene Therapy of Melanoma - The Biologic Paradigm.
Presented: MD Anderson Symposium on Melanoma, February 22-24, 1995.

206. Tahara H, Zitvogel L, Storkus WJ, Robbins PD, Lotze MT. IL-12 gene therapy of cancer: animal models to clinical application.
Presented: International Symposium of Molecular Cell Biology of Macrophages '95. May 18-19, 1995; Japan.

207. Lotze MT. Tumor Immunology and Immunotherapy. Discussion of abstracts.
Presented: American Society of Clinical Oncology; 31st Meeting. May 20-23, 1995; Los Angeles, CA.

208. Mayordomo JI, Storkus WJ, Kast WM, DeLeo AB, Lotze MT. Peptide-pulsed dendritic cells and TAP-deficient cells serve as effective immunogens in tumor vaccines and therapies. Proc. ASCO 14:546(1809).
Presented: American Society of Clinical Oncology; 31st Meeting. May 20-23, 1995; Los Angeles, CA.

209. Zitvogel L, Tahara H, Robbins PD, Davis G, Lotze MT. Cancer gene therapy using a cytokine IL-12 and a costimulatory molecule B7.1. Proc. ASCO 14:226(583).
Presented: American Society of Clinical Oncology; 31st Meeting. May 20-23, 1995; Los Angeles, CA.

210. Lotze MT. Immunotherapy and Gene Therapy in Gynecologic Oncology.
Presented:Treatment of Cancers of Women Conference; June 23-24, 1995; Pittsburgh, PA.

211. Lotze MT. Antigen-specific T-cell responses in University of Pittsburgh tumor vaccine trials.
Presented: Immune Monitoring of Cancer Vaccine Clinical Trials; April 12, 1995; Bethesda, MD.

212. Lotze MT, Tahara H, Storkus WJ, Sitvogel L, Suzuki T, Berman R, Robbins PD. Approaches for IL-12 gene therapy: The non-ãRc T-cell growth factors - cytokine gene therapy for cancer and transplantation.
Presented: IL-12 in Infection: Prospects for Prophylactic and Therapeutic Intervention; May 15-17, 1995; The Cloister; Bethesda, MD.

213. Lotze MT. Vaccine Strategies.
Presented: 95th General Meeting American Society for Microbiology. May 21-25, 1995; Washington Convention Center; Washington, DC.

214. Lotze MT. Genetic Therapy for Cancer: Potential Role of rhIL-12.
Presented: Interleukin Twelve: Clinical Progress and Future Directions. Four Seasons Hotel and Resort; June 3-4, 1995; Irving, TX.

215. Lotze MT. New Regional and Sytemic Treatment for Melanoma.
Presented: Southern Society of Clinical Surgeons. April 10, 1995, Pittsburgh, PA.

216. Lotze MT. IL-4 and IL-12 Gene Therapy
Presented: First PCI Gene Therapy for Cancer Retreat. June 24, 1995, Johnstown, PA.

217. Lotze MT. Gene Therapy of Infectious Diseases and Cancer. Can J Infect Dis. 6:191C, 1995.
Presented: Plenary Session of the 19th International Congress of Chemotherapy, July 17th, 1995, Montreal.

218. Lotze MT. Biologic Therapy of Cancer. A New Role for Dendritic Cells. PCI Grand Rounds; September 1, 1995; Pittsburgh, PA.

219. Couderc F, Amoscato A, Storkus WJ, Hempel JD, Lotze MT. MHC Class I Peptides Identification in Melanoma Cells by Micellar Electrokinetic Chromatography and Laser-induced Fluorescence Detection.
Presented: Eighth Internation Symposium on High Performance Capillary Electrophoresis. January 21-25, 1996; Orlando, FL.

220. Zitvogel L, Storkus WJ, Tahara H, Mayordomo JI, Tjandrawan T, Robbins PD, Lotze MT. Cancer Vaccines Engineered with IL-4/IL-12+B7.1: Towards the Adaptive Immunotherapy Using Genetically Modified Dendritic Cells.
Presented: 2nd European Conference on Gene Therapy of Cancer; King=s College, Strand, London; September 7, 1995.

221. Doughty LA, Patrene K, Boggs SS, Tahara H, Lotze MT, Evans CH, Robbins PD. The effect of constitutive expression of viral IL-10 or soluble TNF receptor (p75) in mice reconstituted with genetically modified bone marrow stem cells on endotoxin induced IL-6 production. Pediatric Research 37(4):44A, 1995.
Presented: Pediaric Research Society.

---

222. Nishihara K, Barth RF, Lang JC, Wilkie N, Oda Y, Kikuchi H, Lotze MT. Enhanced *in vitro* and *in vivo* tumoricidal activities of IFN-ã, IL-4, IL-6, and TNF-á gene transfected macrophages.
Presented: 17th Symposium on the International Association for Comparative Research on Leukemia and Related Disease; Gene Therapy: New Frontiers. Dublin, Ireland; September 18-21, 1994.

223. Goydos JS, Finn OJ, Lotze MT. Induction of specific immune reactivity in patients with adenocarcinomas of the breast, pancreas and colon using a synthetic mucin vaccine in a Phase I trial.
Presented: 29th Annual Meeting of the Association for Academic Surgery, Dearborn, Michigan, November 8-11, 1995.

224. Tahara H, Zitvogel L, Robbins PD, Lotze MT. IL-12 gene therapy using direct injection of tumors with genetically engineered autologous fibroblasts. Gene Therapy 2:674(006), 1995.
Presented: The First Annual Meeting 1995 Japanese Society of Gene Therapy, The University of Tokyo, Bunkyo-ku, Tokyo, Japan, May 21, 1995.

225. Lotze MT, Storkus WJ, Tahara H, Amoscato A, Mayordomo HI, Zitvogel L. Molecular vaccines for cancer - role of IL-12. Immunology 86:Suppl. 1-28(IS42), 1995.
Presented: Joint Congress of the British Society of Immunology and the Nederlandse Vereniging voor Immunologie; Brighton, UK; Dec 6-8, 1995.

226. Finn OJ, McKolanis JR, Nalesnik MA, Clarke MR, Lotze MT, Ochoa AC. T cell defects in

advanced breast, pancreatic, and colon cancer and improvements after vaccination with a mucin peptide. Proc. Amer. Assoc.Cancer Research 37:475 (3344), 1996.
Presented: 1996 Annual AACR Meeting; April 21, 1996; Washington, DC.

227. Robbins PD, Ghivizzani SC, Kang R, Storkus WJ, Tahara H, Zitvogel L, Couderc B, Lotze MT. Development of gene therapies for arthritis and cancer.
Presented:Luneborg Symposium on Interdisciplinary Approaches to Gene Therapy. Luneborg, Germany March 25-27, 1996.

228. McKolanis JR, Pecher G, Lotze MT, Finn OJ. Mucin reactive CTL induced by *in vivo* immunization. Proc. Amer. Assoc.Cancer Research 37:466 (3177).
Presented: 1996 Annual AACR Meeting; April 23, 1996; Washington, DC.

229. Zitvogel L, Mayordomo JI, Tjandrawan T, DeLeo AB, Lotze MT, Storkus WJ.Therapy of poorly immunogenic murine tumors with tumor peptide-pulsed dendritic cells: Induction of a specific antitumor immune response mediated by T-cells, Th1-associated cytokines and B7 costimulation. Proc. Amer. Assoc.Cancer Research 37:473 (3225).
Presented: 1996 Annual AACR Meeting; April 22, 1996; Washington, DC.

230. Mayordomo JI, Loftus JI, Sakamoto DJ, Lotze MT, Storkus WJ, Appella E, DeLeo AB.Therapy of murine tumors with p53 wild type sequence peptide-based vaccine. Proc. Amer. Assoc.Cancer Research 37:475 (3244).
Presented: 1996 Annual AACR Meeting; April 23, 1996; Washington, DC.

231. Dookeran KA, Shipe-Spotloe J, Rubin JT, Sikora SS, Kirkwood JM, Lotze MT, Logan T. Low dose systemic interleukin-2 therapy to patients with associated co-morbid conditions. Proc. Amer. Assoc.Cancer Research 37:488 (3330).
Presented: 1996 Annual AACR Meeting; April 21, 1996; Washington, DC.

232. Tahara H, Zitvogel L, Storkus WJ, Elder EM, Kinzler D, Whiteside TL, Robbins PD, Lotze MT. Phase I clinical trial of interleukin-12 (IL-12) gene therapy using direct injection of tumors with genetically engineereed fibroblasts. Proc. ASCO 15:235 (579), 1996.

233. Atkins M, O'Boyld K, Sosman J, Wiss G, Lotze M, Dutcher J, Fisher R. Prospective randomized trila of lisofylline (LSF) for the modulation of interleukin-2 (IL-2) toxicity. Proc. ASCO 15:273(729).

234. Davis ID, Suzuki T, Tahara H, Robbins P, Narula S, Zavodny P., Lotze MT. Murine interleukin-4 (mIL-4)-based therapeutic tumor vaccines generated by transfection of multiple genes into a poorly immunogenic tumor cell line. Proc. ASCO 15:555(1808), 1996.

235. Storkus WJ, Kirkwood JM, Mayordomo JI, Kinzler D, Johnson C, Tahara H, Elder E, Baar J, Davis J, Paradise C, Shipe-Spotloe J, Lotze MT. Melanoma peptide vaccine: a randomized Phase I evaluation of MART-1, gp100, and tyrosinase peptide vaccines in patients with malignant

melanoma. Proc. ASCO 15:556, 1996.

236. Atkins MB, Robertson M, Gordon MS, Lotze MT, Du Bois J, Ritz J, Sandler A, Edington HD, Sherman ML. Phase I evaluation of intravenous recombinant human interleukin-12 (rHIL-12) in patients with advanced malignancy. Proc. ASCO.15:270(718), 1996.

237. Lotze MT. Gene Therapy for Surgical Cancers. The 1st British Journal of Surgery Lecturer; 84th Meeting of the Surgical Research Society; Birmingham, June 27th, 1996.

238 Lotze MT State of the Art Lecture: Gene Therapy and Clinical Medicine. Update in Internal Medicine: Practice in the New Health Care Environment.
Presented October 30th, 1996; Pittsburgh, PA.

239. Shurin MR, Marakovsky E, Lotze MT. FLT3-ligand inhibits tumor progression in murine model. J. Immunotherapy .
Presented: 11th Annual Society of Biological Therapy Meeting, Washington, DC; October 23-26, 1996.

240. Bozik ME, Lotze MT, Chambers WH. Effective treatment of CNS tumor with IL-4 producing glioma vaccine. J. Immunotherapy .
Presented: 11th Annual Society of Biological Therapy Meeting, Washington, DC; October 23-26, 1996.

241. Berman RM, Rilo HL, Hoehnke C, Shurin MR, Naula SK, Maraskovsky E, Lotze MT. Interleukin-10 effects on in vivo generation and in vivo function of dendritic cells. J. Immunotherapy .
Presented: 11th Annual Society of Biological Therapy Meeting, Washington, DC; October 23-26, 1996.

242. Farhood H, Linsley PSS, Storkus WJ, Lotze MT. Immunopotentiation by CD28 and Interleukin 12. J. Immunotherapy .
Presented: 11th Annual Society of Biological Therapy Meeting, Washington, DC; October 23-26, 1996.

243. Cai Q, Presky D, Robbins PD, Lotze MT, Tahara H. Interleukin-12 (IL-12) expression at the tumor site is criticial in natural tumor rejection but not in mouse IL-10 mediated anti-tumor effects. J. Immunotherapy .
Presented: 11th Annual Society of Biological Therapy Meeting, Washington, DC; October 23-26, 1996.

244. Sikora R, Rilo H, Tahara H, Lotze MT, Carty SE. Viral Interleukin-10 (vIL-10) expression in rat pancreatic islets by bioballistic Gene Transfer. Proc. Assoc. Acad. Surg.30:78(30). 1996.
Presented: 30th Annual Meeting Association for Academic Surgery, November 13th-16th, 1996; Chicago, Ill.

245. Chau Gar-Yang, Dooker5an KA, Brumfield A, Tueting T, Lotze MT, Rubin JT. Effective antitumor activity of interferon alfa plus ethiodol in an animal model. Proc. Assoc. Acad. Surg.30:167(P9). 1996.
Presented: 30th Annual Meeting Association for Academic Surgery, November 13th-16th, 1996; Chicago, Ill.

246. Logan T, Elder E, Whiteside T, Kirkwood J, Lotze M, Friberg D, Hrebinko R, Donnelly S, Becich M, Edington H, Shipe-Spotloe J, Hakala T. Pilot study of *in vitro* sensitization with anti-CD3 (OKT3) stimulated vaccine draining lymph node lymphocytes in patients with metastatic renal carcinoma and melanoma. Proc. ASCO 16:432a (1551),1997.
Presented: ASCO Annual Meeting, May 17-20, 1997; Denver, CO.

247. Dutcher J, Atkins M, Fisher R, Weiss G, Margolin K, Aronson F, Lotze M, Gordon M. IL-2-based therapy in metastatic renal cell cancer (MRCC): Cytokine Working Group (CWG) experience. Proc. ASCO 16:327a (1166),1997.
Presented: ASCO Annual Meeting, May 17-20, 1997; Denver, CO.

248. Tahara H, Zitvogel L, Storkus WJ, Elder EM, Kinzler D, Whiteside TL, Robbins PD, Lotze MT. Antitumor effects in patients with melanoma, head and neck and breast cancer in a Phase I/II clinical trial of Interleukin-12 (IL-12) gene therapy. Proc. ASCO 16:438a (1568),1997.
Presented: ASCO Annual Meeting, May 17-20, 1997; Denver, CO.

249. Atkins M, Lotze M, Wiernik P, Margolin K, Weiss G, Parkinson D, Hawkins M, Paradise C, Rosenberg S. High-dose IL-2 therapy alone results in long-term durable complete responses in patients with metastatic melanoma. Proc. ASCO 16:494a (1780),1997.
Presented: ASCO Annual Meeting, May 17-20, 1997; Denver, CO.

250. Maeurer MJ, Walter W, Trinder P, Seliger B, Lotze MT. Interleukin-7 (IL-7) secretion by epithelial tumors. Potential implications for organ specific immunity.
Keystone Symposia on Cellular Immunology and the Immunotherapy of Cancer III; Copper Mountain, CO; February 1-7, 1997.

251. Robertson MJ, Cameron C, Atkins MB, Gordon MS, Lotze MT, Sherman ML, Ritz J. Immunologic effects of Interleukin-12 administered by bolus intravenous injection to patients with advanced cancer.
Submitted: American Society of Hematology

252. Nishioka Y, Shurin M, Robbins PD, Storkus WJ, Lotze MT, Tahara H. Effective tumor immunotherapy using bone marrow-derived dendritic cells (DC)=s genetically engineered to express Interleukin 12. Journal of Immunotherapy 20:419, 1997.
Presented: 12th Annual Meeting of The Society of Biologic Therapy; Pasadena California October 22-25, 1997.

253. Shurin GV, Esway J, Subbotin VM, Lotze MT, Barksdale EM. Functional Fas ligand is

expressed on neuroblastoma cells. Journal of Immunotherapy 20:403, 1997.
Submitted: 12th Annual Meeting of The Society of Biologic Therapy; Pasadena California October 22-25, 1997.

254. Esway J, Shurin GV, Lotze MT, Barksdale EM. Creating immune privilege: Neuroblastoma soluble factors cause apoptosis of Fas-sensitive targets. Journal of Immunotherapy 20:402, 1997.
Submitted: 12th Annual Meeting of The Society of Biologic Therapy; Pasadena California October 22-25, 1997.

255. Numasaki M, Lotze MT, Tahara H. Interleukin 17 gene transfection into murine fibrosarcoma cell line MCA 205 increases tumorigenicity correlated with enhanced tumor microvascularity. Journal of Immunotherapy 20:399, 1997.
Presented: 12th Annual Meeting of The Society of Biologic Therapy; Pasadena California October 22-25, 1997.

256. Kinzler D, Tahara H, Elder E, Johnson C, Nguyen N, Hanan S, Thomas R, Vu H, Duran P, Kirkwood J, Lotze M. Patient recruitment onto a Phase I clinical trial of Interleukin 12 (IL-12) gene therapy for cancer. Journal of Immunotherapy 20:400, 1997.
Presented: 12th Annual Meeting of The Society of Biologic Therapy; Pasadena California October 22-25, 1997.

---

257. Peron JM, Esche C, Hunter O, Subbotin VM, Lotze MT, Shurin MR. Effective treatment of murine liver metastases using FLT3 ligand (FL) and IL-12. Journal of Immunotherapy 20:400, 1997.
Presented: 12th Annual Meeting of The Society of Biologic Therapy; Pasadena California October 22-25, 1997.

258. Hunter O, Haluszczak C, Subbotin VM, Lfotze MT, Shurin MR. Administration of IL-12 and FLT3 ligand enhances murine dendritic cell generation. Journal of Immunotherapy 20:401, 1997.
Presented: 12th Annual Meeting of The Society of Biologic Therapy; Pasadena California October 22-25, 1997.

259. Baar J, Lotze MT. Interferon gamma inducible cytokine gene expression in plasmid-transfected dendritic cells. Journal of Immunotherapy 20:403, 1997.
Presented: 12th Annual Meeting of The Society of Biologic Therapy; Pasadena California October 22-25, 1997.

260. Esche C, Shurin MR, Haluszczak C, Peron JM, Lotze MT. Generation of human dendritic cells from CD34+ precursors for human clinical trials. Journal of Immunotherapy 20:403, 1997.
Presented: 12th Annual Meeting of The Society of Biologic Therapy; Pasadena California October 22-25, 1997.

261. Shurin MR, Esche C, Lokshin A, Lotze MT. Tumors induce apoptosis of dendritic cells in vitro. Journal of Immunotherapy 20:403, 1997.

Presented: 12th Annual Meeting of The Society of Biologic Therapy; Pasadena California October 22-25, 1997.

262. Hall TD, Kinzler DM, Elder EM, Whiteside TL, Storkus WJ, Lotze MT. Evaluation and response of patients immunized to HLA-A2 polyepitope peptide mixture in patients with metastatic melanoma using autologous dendritic cells cultured with IL-4 and GM-CSF. Journal of Immunotherapy 20:405, 1997.
Presented: 12th Annual Meeting of The Society of Biologic Therapy; Pasadena California October 22-25, 1997.

263. Campbell RL, Repasky EA, Lotze MT. T-cell spectrin (fodrin) aggregation and uropod formation following dendritic cell encounter. Journal of Immunotherapy 20:405, 1997.
Presented: 12th Annual Meeting of The Society of Biologic Therapy; Pasadena California October 22-25, 1997.

264. Haluszczak C, Lotze MT, Shurin MR. IL-12 and FLT3 ligand differentially stimulate lymphoid and myeloid dendropoiesis in vivo. Journal of Immunotherapy 20:406, 1997.
Presented: 12th Annual Meeting of The Society of Biologic Therapy; Pasadena California October 22-25, 1997.

265. Elder EM, Lotze MT, Whiteside TL. Culture of human dendritic cells (DC) for the therapy of patients with cancer. Journal of Immunotherapy 20:407, 1997.
Presented: 12th Annual Meeting of The Society of Biologic Therapy; Pasadena California October 22-25, 1997.

266. Elder EM, Stolinski LA, Whiteside TL, Lotze MT. Changes in DC precursor numbers during in vivo IL-2 adminstration. Journal of Immunotherapy 20:407, 1997.
Presented: 12th Annual Meeting of The Society of Biologic Therapy; Pasadena California October 22-25, 1997.

267. Kadakia MP, Birkenbach M, Baar J, Farhood H, Lotze MT. Modulation of interleukin-12 by Epstein Barr Virus induced gene production. Journal of Immunotherapy 20:411, 1997.
Presented: 12th Annual Meeting of The Society of Biologic Therapy; Pasadena California October 22-25, 1997.

268. Tahara H, Elder EM, Cai Q, Kinzler DM, Storkus WJ, Zitvogel L, Kirkwood JM, Whitesdie, Robbins PD, Lotze MT. Phase I clinical trial of Interleukin-12 cytokine gene therapy with objective responses in cancer patients with various histologies. Journal of Immunotherapy 20:416, 1997.
Presented: 12th Annual Meeting of The Society of Biologic Therapy; Pasadena California October 22-25, 1997.

269. Osaki T, Péron M.-M., Cai Q, Okamura H, Robbins PD, Kurimoto M, Lotze MT, Tahara H. . Analyses of antitumor effects of interferon-ã-inducing factor/Interleukin-18 against murine

tumors. Journal of Immunotherapy 20:417, 1997.
Presented: 12th Annual Meeting of The Society of Biologic Therapy; Pasadena California October 22-25, 1997.

270. Lotze MT. Dendritic Cell Therapy of Cancer.
Presented: 12th Annual Meeting of The Society of Biologic Therapy; Pasadena California October 22-25, 1997.

271. Lotze MT. Life and death in the Fas Lane:Immune Effectors in the Tumor Microenvironment.
Presented: 12th Annual Meeting of The Society of Biologic Therapy; Pasadena California October 22-25, 1997.

272. Lotze MT.Therapy of Cancer with Dendritic Cells.
Presented: Immunologic Attacks on Cancer, Cold Spring Harbor and the Banbury Center; October 19-22, 1997.

273. Lotze MT. Regional Therapy of Cancer.
Presented: University of Hawaii at Manoa; October 27, 1997.

274. Lotze MT. Apoptosis in the tumor microenvironment.
Presented: University of Sapporo; Sapporo, Japan; October 28, 1997.

275. Lotze MT. IL-12 transfected fibroblasts or DC=s for cancer therapy.
Presented: The 27th Annual Meeting of the Japanese Society for Immunology; Sapporo, Japan; October 29, 1997.

276. MT Lotze, T Osaki, J-M Peron, M Shurin, J Baar, H Tahara. Cytokines which induce Interferon-gamma (IFN-α) - IL-2, IL-12, and IL-18: Regulation of tumor immunity. J. Leucocyte Biology
Presented: 32nd Annual Meeting of the Society for Leucocyte Biology; Baltimore, MD; December 4-7, 1997.

277. Orucevic A, Green AM, Lotze MT, Billiar TR. Nitric oxide induces apoptosis and enhances FAS expression in murine tumor cell lines. Proc Amer Assoc Cancer Res 39:580(3947),1998
Presented:AACR Annual Meeting March 28-April 1, 1998.

278. Farhood H, Hershberger P, Shurin G, Barksdale E, Lotze MT. Ubiquitous intracellular Fas ligand expression by human and mouse tumor cells. Proc Amer Assoc Cancer Res 39:84(569),1998
Presented:AACR Annual Meeting March 28-April 1, 1998.

279. Thomas RL, Lotze MT, Amoscato AA. Identification of oxidized ceramide as the predominant mass spectrometric identified cellular lipid increased in tumor cells undergoing apoptosis.
Presented: FASEB/AAI Annual Meeting, April 19, 1998, San Francisco.

280. M Atkins, J Dutcher, M Gordon, T Goss, T Logan, M Lotze, K Margolin, T Plasse, J Sosman, G Weiss Quality of life (QOL) assessment of patients with renal cancer treated with interleukin-2, interferoná and 5-fluorouracil.
Presented: Annual ASCO Meeting.

281. Agarwala SS, Von Oosterom A, Petruzelli G, Johnson JT, Fritz MA, Horowitz JA, Rybak ME, Burke E, Van de Bogaert, Lotze MT. Phase I study of rAd/p53 in patients (pts) with advanced head and neck cancer (HNC).
Presented: Annual ASCO Meeting.

282. Dookeran KA, Hanan SH, Lotze MT. Effective regional therapy of cancer with sustained-release nitric oxide-donor, nitroprusside in ethiodol.
Presented: 51st Annual Society of Surgical Oncology Meeting, San Diego, March 26-29, 1998.

283. Flaherty LE, Atkins M, Clark J, Dutcher J, Gordon M, Lotze M, Margolin K, Sosman J, Weiss G. Randomized Phase II trial of chemotherapy and outpatient biotherapy with Interleukin-2 (IL-2) and interferon alpha (IFN) in metastatic malignant melanoma (MMM).
Presented: Annual ASCO Meeting.

284. Flaherty LE, Atkins M, Clark J, Dutcher J, Gordon M, Lotze M, Margolin K, Sosman J, Weiss G. Randomized Phase II trial of chemotherapy and outpatient biotherapy in metastatic malignant melanoma (MMM). Cancer Investigation 16:S1, p28-29, 1998.
Presented: New York Chemotherapy Symposium.

285. Chikamatsu K, Whiteside TL, Storkus W, Lotze MT and DeLeo AB. Generation of human cytotoxic T lymphocytes recognizing wild-type sequence p53 epitopes from peripheral blood lymphocytes using autologous dendritic cells. Proc Amer Assoc Cancer Res 39:84(569),1998
Presented:AACR Annual Meeting March 28-April 1, 1998, New Orleans.

286. Esche C, Lotze MT, Shurin MR. Accumulation of intratumoral dendritic cells in vivo using FLT3 ligand: a new tool for immunotherapy of melanoma and lymphoma. Proc Amer Assoc Cancer Res 39:172(1180),1998
Presented:AACR Annual Meeting March 28-April 1, 1998, New Orleans.

287. Shurin MR, Lokshin A, Esche C, Lotze MT. Tumors induce apoptosis of dendritic cells:role of FLT3-ligand and IL-12 in tumor -associated dendritic cell function. Proc Amer Assoc Cancer Res 39:550(3745),1998
Presented:AACR Annual Meeting March 28-April 1, 1998, New Orleans.

288. Giezeman-Smits KM, Okada H, Brisette-Storkus CS, Villa LA, Attanucci J, Tahara H, Lotze MT, Chambers WH, Bozik ME. Immunity aftainst Interleukin-4 producing 9L rat glioma is mediated by tumor-specific interferon-ã producing CD8+ cells. FASEB J. 12:1622(A278), 1998.
Presented: FASEB/AAI Annual Meeting, April 19, 1998, San Francisco.

289. Péron JM, Subbotin V, Haluszczak C, Lotze MT, Shurin MR. The anti-tumor effect of FLT3-ligand administration on murine liver metastases is mediated in part by NK cells. Digestive Diseases Week.Abstract 1937 (pA-482), 1998.
Presented:Digestive Diseases Week, May 18, 1998, New Orleans.

290. Lotze MT. Immunotherapy of Unresectable Pancreatic Carcinoma. Digestive Diseases Week and the 98th Annual Meeting of the American Gastroenterologic Association. 1998 Consensus Conference Program.
Presented:Digestive Diseases Week, May 18, 1998, New Orleans.

291. Lotze MT. Dendritic cell therapies.
Presented ASCO Education Session on Tumor Vaccines. Saturday May 16, 1998 and Sunday May 17, 1998., Los Angeles.

292. Lotze MT. Dendritic Cell Based Therapy of Cancer.
Presented at the 10th British Cytokine Group Meeting in London; June 18th, 1998.

293. Lotze MT, Ronna Campbell, Andrew Amoscato, Robbie Mailliard, Walter J. Storkus, Hideaki Tahara, Michael Shurin."Dendritic cells Regulate the Immune Response to Cancer"
Presented: 13th Aspen Cancer Conference July 19th, 1998.

294. Odoux C, Lotze MT, Kim PK, Amoscato A, Luketich J, Weigel T. Paclitaxel activates caspase-3 and induces apoptotic death in human lung cancer: Is it really fas dependent?
Presented: Submitted American Thoracic Society, April 23-28, 1999.

295. Dallal R, Christakos P, Lotze MT. Paucity of dendritic cell (DC) infiltrate in pancreatic carcinoma (PCa) predicts poor prognosis. Proc.Assoc of Acad. Surgery 32:207(P7), 1998.
Presented: Association of Academic Surgery Seattle November 19-22, 1998.

296. Lotze MT. Genetically Modified Dendritic Cells as Vaccines.
Presented:Postgraduate Course No. 15 - Molecular Biology for Surgeons: A Primer. 84th Annual Clinical Congress of theAmerican College of Surgeons, Orlando FL; October 27, 1998.

297. Shurin MR, Esche C, Peron J-M, Hunter OC, Lotze MT. FLT3 Ligand (FL) and Interleukin-12 (IL-12) regualte generation, migration, and survival of dendritic cells (DC). Eur. Cytokine Network 9:509 (#229).
Presented: International Cytokine Conference Jerusalem, September 1998.

298. Lotze MT. Enhancement of Immune Response by Cytokines.
Presented: Keystone Symposium, HIV Vaccine Development: Opportunities and Challenges. Keystone, CO. January 8, 1999.

299. Lotze MT. Angiogenesis and the Dendritic Cell System.
Presented: First International Symposium on Anti-Angiogenic Agents. Irving, TX. January 29, 1999.

300. Lotze MT. Direct Delivery of Dendritic Cells to Tumors.
Presented: International Workshop on the Use of Dendritic Cells in Cancer Therapy. Innsbruck, Austria. February 11, 1999.

301. Lotze MT. Recognizing Melanoma – evaluation and treatment.
Presented: Citizens General Hospital, New Kensington, PA. March 9, 1999.

302. Lotze MT. Cytokine Gene Therapy of Cancer.
Presented: Cancer Progress Conference March 22-23, 1999. The Plaza, New York City.

302. Lotze MT. Tumor Vaccines
Presented: American Association for Cancer Research 90th Annual Meeting. Philadelphia, PA. April 10-14, 1999.

303. Lotze MT. The Role of Dendritic Cells in Cancer Vaccines.
Presented: Fifth International Congress on Biological Response Modifiers. Toronto, Canada. April 29-30, 1999.

304. Egawa S. Lotze MT. Future Directions for the Biologic Therapy for Pancreatic Cancer.
Presented: Innovations in GI Cancer Control and Management: Genetics and Pancreatic Cancer. Pittsburgh, PA. May 3, 1999.

305. Lotze MT. Melanoma Vaccines
Presented: Hematology/Oncology Noontime Conference. Darthmouth-Hitchcock Medical Center. Lebanon, NH May 13, 1999.

306. Flaherty LE, Atkins M, Sosman HJ, Clark J, Margolin K, Dutcher J, Gordon M, Lotze M, Weiss G.Randomized Phase II Trial of Chemotherapy and outaptient biotherapy with Interleukin-2 (IL-2) and interferon alpha (IFN) in metastatic malignant melanoma (MMM).
Presented: ASCO, May 14, 1999.

306. Kang W-K, Park C, Lee H, Jang S, Choi K, Kim WS, Yoon SS, Lee H-K, Yang J-Y, Lotze MT, Robbins PS, Tahara H, Kim S, Park K, Park CH. A Phase I Clinical Trial of Gene Therapy with Interleukin-12 Gene-modified Autologous Fibroblasts: Monitoring of the Immune Response.
Presented: American Society of Gene Therapy; Washington, DC. June 11, 1999.

307. Lotze MT. Into Thin Air – Regulation of the Acute and Chronic Inflammatory Response by Interferon γ Inducting Cytokines.
Presented: British Cytokine Group - Perspectives in Cytokine Research. London, UK. June 18-19, 1999.

308. Lotze MT. Advances in Vaccine Therapy for Cancer.
Presented: Surgery Branch/ACS Research Seminar Program. Bethesda, MD. August 27, 1999.

309. Lotze MT. Cytokine Gene Therapy of Cancer Using Fibroblasts or DCs.
Presented: 1999 Meeting of the Institute of Human Virology. Baltimore, MD. September 2, 1999.

310. Mailliard RB, Lotze MT. Major Phenotypic, Morphologic and Functional Differences between DCs Stimulated with Monocyte-condition Medium or Cytokine Cocktail.
Presented: Tumor Escape from Immune Recognition: Molecular Mechanism and Functional Significance Meeting. Baltimore, MD. August 22-23, 1999.

311. Lotze MT. Summary: Antitumor Response in RCC
Presented: First International Kidney Cancer Symposium. Chicago, IL. October 2, 1999

312. Lotze MT. Protection of Effector Cells in the Tumor Microenvironment: DC, Vaccines, Cytokines.
Presented: International Cancer Microenvironment Forum. Pittsburgh, PA. October 4-5, 1999.

313. Lotze MT. Dendritic Cell Therapy: Not Just an Antigen Presenting Cell.
Presented: 6th International Workshop on Langerhans Cells. New York, NY October 9, 1999.

314. Lotze MT. Dendritic Cell Therapy of Cancer
Presented: Surgical Biology Club 47th Annual Meeting. San Francisco, CA October 10, 1999.

315. Lotze MT. Novel Cytokines in the Treatment of Cancer.
Presented: AACR-NCI-EORTC International Conference. Molecular Targets and Cancer Therapeutics. Washington, DC. November 16, 1999.

316. Lotze MT. Dendritic Cells – not just antigen-presenting cells.
Presented: DC Approaches to Immunotherapy. Boston, MA October 31, 1999

317. Numasaki M, Nukiwa T, Robbins D, Lotze MT, Tahara H. CD40L expression by tumor cells elicits potent anti-tumor immunity: IL-12 dependent and IFN-γ-independent mechanism and in cooperation with GMCSF and IL-12. Proc. AACR 91:114 (#725),2000
Presented: AACR 91st Annual Meeting April 1-5, 2000, San Francisco, CA

318. Okada H, Attanucci J, Witham T, Villa L, Erff M, Fellows WK, Lotze MT, Chambers WH, Pollack IF. Effective combination therapy using a dendritic cell vaccine and local interleukin-4

gene delivery for gliomas. Proc. AACR 91:114 (#727),2000
Presented: AACR 91st Annual Meeting April 1-5, 2000, San Francisco, CA

319. Dworacki G, Meidenbauer N, Kuss I, Saito T, Hoffmann T, Gooding W, Lotze M, Whiteside TL. Tumor-induced signaling defects and apoptosis in circulating T cells of patients with metastatic melanoma. Proc. AACR 91:711(#4522),2000
Presented: AACR 91st Annual Meeting April 1-5, 2000, San Francisco, CA

320. Pirtskhalaishvili G, Gambotto A, Yamabe K, Lotze MT, Shurin MR. Protection of dendritic cells (DC) from tumor-induced apoptosis increases the efficacy of DC-based therapy in a murine prostate cancer model. Proc. AACR 91:43(#277),2000
Presented: AACR 91st Annual Meeting April 1-5, 2000, San Francisco, CA

321. Egawa S, Mailiard R, Lotze MT. Peripheral blood monocytes derived dendritic cells generated with interferon-α and granulocyte-macrophage cology-stimulating factor are potent IL-12 producers. Proc. AACR 91:191(#1223),2000
Presented: AACR 91st Annual Meeting April 1-5, 2000, San Francisco, CA

322. Esche C, Cai Q, Subbotin VM, Lotze MT, Shurin MR. Interleukin-12 stimulates predominantly myeloid dendropoiesis while FLT3 ligand stimulates both myeloid and lymphoid dendropoiesis in vivo. Proc. AACR 91:375(#2378),2000
Presented: AACR 91st Annual Meeting April 1-5, 2000, San Francisco, CA

323. Shurin GV, Shurin MR, Lotze MT, Barksdale EM. Neuroblastoma-derived gangliosides inhibit dendritic cell generation and function. Proc. AACR 91:415(#2644),2000
Presented: AACR 91st Annual Meeting April 1-5, 2000, San Francisco, CA

324. Shurin MR, Lotze MT. A new mechanism of antitumor activity of IL-12: Stimulation of the dendritic cell system. Proc. AACR 91:521(#3321), 2000
Presented: AACR 91st Annual Meeting April 1-5, 2000, San Francisco, CA

325. Lotze MT. Molecular Targets for Melanoma Therapy; invited speaker inAACR Minisymposia on Melanoma (Chair: Nicholas Hayward)
Presented: AACR 91st Annual Meeting April 1-5, 2000, San Francisco, CA

326. Shurin MR, Esche C., Galaychuk I, Lotze MT. Melanoma-induced inhibtion of the dendritic cell system. Proc. ASCO 36: 556a(#2189),2000
Presented: ASCO 36th Annual Meeting May 20-23, 2000, New Orleans, LA

327. Jonak ZL, Ho YS, Trulli S, McCabe FL, Maier C, Kirkpatrick R, Elefante L, Chen Y-J, Herzyk D, Johansen K, Lotze MT, Johnson RK. IL18 mediates antitumor effects in advanced murine tumor models. J. Immunotherapy, in press.
Presented: SBT 15th Annual Scientific Meeting, Seattle October 28-31, 2000.

328. Shimamura H, Cumberland R, Hiroishi K, Watkins SC, Lotze MT, Baar J. Murine dendritic cells induce the apoptosis of a syngeneic tumor. J. Immunotherapy, in press.
Presented: SBT 15th Annual Scientific Meeting, Seattle October 28-31, 2000.

329. Son Y-I, Mailliard RB, Myers EN, Lotze MT. Dendritic cells with apoptotic tumors have antitumor effects when combined with IL-2. J. Immunotherapy, in press.
Presented: SBT 15th Annual Scientific Meeting, Seattle October 28-31, 2000.

330. Mailliard RB, Egawa S, son Y-I, Bahnson A, Lotze MT. Dendritic cells and T-cell interactions following specific engagement of tumor. J. Immunotherapy, in press.
Presented: SBT 15th Annual Scientific Meeting, Seattle October 28-31, 2000.

331. Zdenka L Jonak, Stephen Trulli, Francis L McCabe, Danuta Herzyk, Robert Kirkpatrick, Louis Elefante, Yi-Jiun Chen, Kyung Johanson, Curtis Maier , Yen Sen Ho, Michael T Lotze, Randall K Johnson. IL-18 is an Immunomodulator of Anti-tumor Response in Advanced Murine Tumor Models
Presented: AACR, New Orleans, 2001.

332. Fujii S-I, Shimizu K, Shimizu T, Lotze MT. Survival of antigen specific CD8+ T cells capable of mediating rapid effector function and tumor elimination.
Pesented: IL10 Meeting, 2001; Milan, Italy.

---

333. Lotze Michael T. Novel Biologic Approaches in Lung Cancer.
Presented: 2nd International Lung Cancer Congress. Kauai, Hawaii; July 18-21, 2001.

334. Sallusto Federica and Lotze Michael T. Workshop 2.7. Origin and Migration of Dendritic Cells.
Presented: 11th International Congress of Immunology. 22-27 July 2001; Stockholm, Sweden.

335. Lotze Michael T. Interleukin 18 and the regulation of Immunity.
Presented: Trudeau Institute; February, 2001. 100 Algonquin Avenue; Sararanac Lake, NY. 12983.

336. Lotze Michael T. Interleukin 18 promotes immune response to cancer.
Presented: Surgery Branch, NCI. Bethesda MD; August 29, 2001.

337. Lotze Michael T. Dendritic cells mediate cytokine dependent antitumor effects.
Presented: German Immunology Society Meeting, September 29, 2001.

338. Agha-Mohammadi Siamak, Lotze Michael T. Second generation tetracycline-regulated promoters.
Presented: 5th Annual Society of Gene Therapy. June 5-9, 2002; Molecular Therapy 5:S28[76], 2002.

339. Jonak ZL, Trulli S, McCabe FL, Chen Y-J, Elefante L, Tapley P, Herold K, Kirkpatrick R,

Johanson K, Herzyk D, Lotze MT, Johnson RJ. Interleukin-18 has curative activity in advanced murine tumors: Association with T/NK/DC cellular infiltrates.
Presented: 93rd Annual Meeting of the American Association for Cancer Research, San Francisco CA, April 6-10, 2002. Proc Amer Assoc Cancer Res 43:438 [2178], 2002.

340. Lotze Michael T. The message is in the medium: Dendrikines including IL-18 regulate immunity.
Presented: First Cellular Therapy Speaker, American Red Cross-Holland Laboratories; Rockville, MD; May 29, 2002.

341. Lotze Michael T. Interleukin 18 Regulates the Immune Response - Preclinical Studies.
Presented: University of Western Australia, June 11, 2002.

342. Lotze Michael T. The Acute and Chronic Immune Response in Cancer - Views of a DC Chauvanist [Evangelist]
Presented: Western Australia Branch, Austalasian Society of Immunology, June 12, 2002

343. Lotze Michael T, Robbie B. Mailliard, Fumiaki Tanaka, Hideaki Tahara, Andrea Gambotto, Sanjay Kumar, Pawel Kalinski. DC-Activating Function of the IL-1 Homologues [IL-1, IL-18, IL-1F7] and NK Cells in the Development of Antitumor Responses.
Presented: 17th Annual Society for Biological Therapy; San Diego; November 9, 2002.

344. Lotze Michael T. Integrating NK and DC into Cancer Therapy. Keynote Speaker.
Presented:6th Annual Regional Biotherapy Symposium, February 22, 2003.

345. Lotze Michael T and Bartlett D. Surgical Forum III; Cochair and Speaker
Presented 53rd Society of Surgical Oncology Meeting; Los Angeles, CA; March 8; 2003.

346. Lotze Michael T. Identifying and Integrating Biomarkers and Surrogates into the Biologic Therapy of Cancer
Presented: Cancer Progress 2003; NY, NY March 17-18, 2003.

347. Lotze Michaet T. Coorganizer, Biologic Therapy of Cancer Minisymposium, May 13-15, 2003;
Presented: 3rd Annual Meeting Federation of Clinical Immunology Societies, Paris.

348. Lotze Michael T. Biologic Therapy of Cancer – Dealing with Death.
Presented: 104th Meeting of the Japan Surgical Association: Challenges for The New Surgical Frontier; Osaka, Japan; April 8, 2004.

349. Lotze Michael T. Biomarkers and Surrogates.
Presented: 2nd Annual Biomarkers and Pharmaco-proteomics; Speeding Drug Discovery and Development; Renaissance Hotel, Washington DC; July 15-16, 2003.

350. Lotze Michael T. Cytokine-based trials for cancer.

Presented: Grand Rounds and Plenary Speaker, Frye-Halloran Symposium [Viruses, Cancer and Immunity] in Neuro-Oncology; Neurosciences Grand Rounds, Massachusetts General Hospital

351. Lotze Michael T. Cytokine Biology and Therapy.
Presented: Cancer Immunology and Immunotherapy Summer School; September 8-13, 2003; Ionian Village, Greece; Organizing Committee and Lecturer

352. Lotze Michael T. Cancer Biometrics; Imaging and Flow Cytometry; Addicted to Death – HMGB1 Promotes Tumor Growth.
Presented: 18th International Meeting, iSBTc; Bethesda, MD. October 30- November 2, 2003.

353. Lotze Michael T. Biomarkers in Melanoma.
Presented: 2èmes Journèes des Cordeliers ; October 1-3, 2003. Paris

354. Lotze Michael T. Etiologic Role of UV and Molecular Markers of Melanoma
Presented: Perspective in Melanoma VI; November 13-14, 2003. Miami

355. Lotze Michael T. Integrating NK and DC into Cancer Therapy.
Presented: Cornell University, Weill Medical College Medicine Grand Rounds; December 4, 2003. New York.

356. Lotze Michael T., DeMarco Richard. HMGB1 and Tumor Immunology
Presented: Symp. VIII Moderator, Biology and Therapeutic Application of DCs in Chronic Inflammation; 6th Congress - Trauma, Shock, Inflammation and Sepsis; March 3, 2004; Munich; Shock 21:S36, 143, 2004.

357. Lotze Michael T. Biologic Therapy of Cancer
Presented: 2004 International Research Forum; Leukemia Research Fund, March 16, 2004. London.

358. Lotze Michael T., Tahara H, Storkus WJ, Whiteside TL, Hall T, Kinzler D, Zeh HJ. Ten Year Experience and Follow-up of Effective Gene Therapy in Patients with Cancer.
Presented: 57th Annual Cancer Symposium; Society of Surgical Oncology; March 17-24, 2004; New York.

359. Lotze Michael T., Biologic Therapy of Cancer by Inhibiting Necrotic Signals: HMGB1.
Presented: Keystone Symposia on New Advances in Drug Discovery; March 21-26, 2004; Coorganizers, Christine M Debouck, Hoshua Boger, Paul A Bartlett.

360. Lotze Michael T. Melanoma is a Disorder of Cell Death.
Presented: 1st International Symposium on Melanoma and other Cutaneous Malignancies; April 23-25, 2004, NYC.

361. Lotze Michael T, Pawel Kalinski, Per Basse, William Chambers, Ronald B. Herberman. Integrating NK and DC into Cancer Therapy.
Presented: 8th Annual Meeting of the Society of Natural Immunity and 20th Natural Killer Cell Workshop; Noordwijkerhout, The Netherlands, April 24-28, 2004.

362. Lotze Michael T. New perspectives on cancer vaccines: lessons for viruses.
Presented: 10th National Symposium on Basic Aspects of Vaccines; Walter Reed, Bethesda MD, April 29, 2004.

363. Lotze Michael T. Biomarkers – Application to drug development, biochemical pathway elucidation, and molecular diagnostics. [VTC]
Presented: Analytic Division of the Analytical Biosciences Group and the Midlands Regiona in conjunctin with the British Mass Spectrometry Society; Burleigh Court; University of Loughborough; Royal Society of Chemistry; May 11 and 12, 2004.

359. Lotze Michael T. Biologic Therapy of GI Tumors
Presented: Yokohama, Japan; World Congress of International Society for Digestive Surgery; December 8-11, 2004.

360. Lotze Michael T. Dying Dangerously: Apoptosis, Necrosis, and Cancer.
Presented: Medical Grand Rounds University of Wisconsin; Madison, WI; Oct. 15, 2004.

361. Lotze Michael T. Ethics of Translational Research
Presented: Clinical Scholars Progra, University of Arizona; Tucson, AZ; Dec. 2, 2004.

362. Lotze Michael T. IL-1 Family Members and HMGB1 Regulate Immunity
Presented: Immunology Grand Rounds, University of Arizona; Tucson, AZ; Dec. 3, 2004

363. Lotze Michael T. Dying Dangerously: Apoptosis, Necrosis, and Cancer.
Presented: Oncology Grand Rounds, University of Arizona; Tucson, AZ; Dec. 3, 2004.

364. Lotze Michael T. Dying Dangerously: Apoptosis, Necrosis, and Cancer.
Presented: TGEN Visiting Scholars Program, Phoenix, AZ; Dec. 6, 2004.

365. Lotze Michael T. Inflammation and necrosis promote cancer.
Presented: Cancer Symposium on Inflammation and Cancer, Genova; Dec. 13, 2004.

366. Lotze Michael T. Chairman Immunological Therapies for Autoimmunity; FOCIS 5th Meeting, Boston; May 2005.

367. Lotze Michael T. Chairman Symposium on Biomarkers and Surrogates for the Surgeon; Society of Surgical Oncology, Atlanta Meeting, March 2005.

368. Lotze Michael T. Jack Kent Cooke Masters Seminar: Sex, Death, and Cytokines. July, 2005

369. Lotze Michael T. ASCO/AACR Methods in Clinical Cancer Research; 2005 Workshop July 30-August 2005. Tips on Getting your Clincial Trials Approved and Implemented; Successful Grantsmanship.

370 Lotze Michael T. HMGB1, A Tumour Derived Endokine: Implications for the immunotherapy of cancer. European Molecular Biology Organization Workshop on Innate Danger Signals and HMGB1. Milan, Italy February 8-11, 2006.

371. Lotze Michael T. Dying Dangerously. DAMPS and Cancer. Preesented.SRI Innate Immunity World Congress, San Diego CA March 20-22, 2006.

372. Lotze MT. DAMPs and Arthritis. 4th Conference on Targeted Therapy; Sorrento, Italy. April 5, 2006.

372. Tsung A, , John J Fung, MD, PhD 3 , Michael T Lotze, MD 4 , Shiguang Qian, MD 2 and Lina Lu MD The World Transplantation Congress 2006. Tolerogenic activity of liver dendritic cells is regulated by TLR4/B7-H1 pathway

EN.REFLIST

IL-12 in Conjunction with Dendritic Cells Enhances Antiviral CD8+ CTL Responses In Vitro

Nina Bhardwaj,*‡ Robert A. Seder,§ Anita Reddy,* and Mary V. Feldman*

**The Rockefeller University, New York 10021; the §Hospital for Special Surgery, New York 10021; and the ‖National Institutes of Health, Bethesda, Maryland 20892*

Abstract

CD8+ cytolytic T lymphocytes (CTLs) are important mediators for resistance to infections and malignant diseases. IL-12 enhances proliferative and cytolytic responses by killer cells, but its function in the generation of human antiviral CD8+ T cell responses has not been defined. We therefore evaluated the role of IL-12 in the generation of CTLs to influenza-infected dendritic cells. IL-12 was not detectable in supernatants of infected-dendritic cells, or during CTL generation. Furthermore, anti–IL-12 antibody did not block CTL generation. However, exogenous IL-12 (30–300 pg/ml) enhanced CD8+ T cell proliferative and cytolytic responses. The effect was greatest in individuals with weak reactivity to influenza virus or at antigen-presenting cell (APC):T cell ratios of 1:100 or less. IL-12 augmented interferon-γ production during CTL generation. The CTL enhancing effects of the cytokine, however, could not be blocked by neutralizing anti–interferon-γ antibody. Together with IL-12, antigen-pulsed dendritic cells may be a useful approach for boosting CTL responses against infectious agents and malignancies. (*J. Clin. Invest.* 1996. 98: 715–722.) Key words: dendritic cells • cytolytic T cells • CD8+ T cells • interleukin 12 • vaccines

Introduction

IL-12 is a heterodimeric cytokine with multiple immunoregulatory activities (1, 2). A critical component of the host's innate immune response to infection, IL-12 is produced early during the inflammatory response by macrophages. It enhances natural killer (NK)[1] cell cytoxicity severalfold and rapidly induces the production of IFNγ, the latter enhancing the antimicrobial activity of phagocytic cells (3–6). IL-12 is also a crucial participant in the development of acquired immune responses. It induces the differentiation of Th1 cells through its ability to prime naive Th0 cells for high IFNγ production (7, 8). Furthermore, it is a potent growth factor for CD8+ T cells (7) and enhances cytolytic responses to alloantigens (9–11) and in anti-CD3 redirected assays (12).

Here, we have evaluated the role of IL-12 in generating cytolytic T lymphocyte (CTL) responses to influenza virus, which continues to be a source of significant morbidity and mortality in humans. We have previously shown that potent human CD8+ CTL responses to live or nonreplicating heat-inactivated influenza virus can be generated from freshly isolated blood T cells, provided dendritic cells are the antigen-presenting cells (APCs) (13, 14). The response is independent of CD4+ T cells or exogenous cytokines. Small numbers of dendritic cells suffice, whereas other APCs (macrophages, B cells) are inactive in this regard. Dendritic cells effect the development of Th1 cells from Th0 cells by the production of IL-12 (15), suggesting that IL-12 is a key component by which these APCs induce T cell–mediated immune responses.

Using the influenza virus system as a model, we show that dendritic cells induce potent CTL responses without producing significant levels of IL-12 endogenously. However, the exogenous application of IL-12 significantly enhances these responses, especially when the baseline CTL response of the donor is poor, or if the number of dendritic cells is limiting. Based on these findings we suggest that IL-12 may be a useful adjuvant for the induction of CTL responses, especially when delivered concomitantly with dendritic cells.

Methods

Culture medium

RPMI-1640 (Gibco Laboratories, Grand Island, NY) supplemented with 20 ug/ml gentamicin, 5% human serum, and 10 mM Hepes buffer.

Blood mononuclear cells

T cells. PBMCs were isolated from buffy coats by density gradient centrifugation over Ficoll Hypaque. T cell–enriched and T cell–depleted (ER-) fractions were prepared by sheep erythrocyte rosetting. T cells were further purified by removal of monocytes, NK cells, and MHC class II+ cells by panning with antibodies to CD11b, CD16, and DR, as described (13). T cell subsets (CD4+ and CD8+) were negatively selected by incubation with Leu 2 or Leu 3 mAbs, followed by panning onto plastic plates coated with goat anti-mouse IgG (13).

Antigen presenting cells. Monocytes were obtained from ER- cells by adherence to plastic for 90 min. The monocytes were dislodged and used as targets in ^{51}Cr release assays (see below). Nonadherent ER- cells were depleted of residual monocytes by panning on gamma globulin–coated dishes. The remaining cells (primarily B cells and dendritic cells) were adequately enriched for dendritic cells to induce strong CTL responses (13). Since B cells pulsed with influenza virus do not elicit CTL responses, in part because the B cell is not infected with influenza virus (13), we used mixtures of B cells and dendritic cells as APCs in some experiments. Highly purified dendritic cells (> 75%) were obtained as low density cells by metrizamide gradient centrifugation, as previously described (13).

Virus preparation

Influenza A virus (PR8, Puerto Rico/8/34, source: allantoic fluid; Spafas Inc., Storrs, CT) was either live, or inactivated at 56°C for 30 min in a water bath. Nonreplicating, heat-inactivated virus, which re-

Address correspondence to Nina Bhardwaj, The Rockefeller University, 1230 York Avenue, New York, NY 10021. Phone: 212-327-8332; FAX: 212-327-8875; E-mail: bhardwn@rockvax.rockefeller.edu

Received for publication 18 March 1996 and accepted in revised form 23 May 1996.

1. *Abbreviations used in this paper:* APC, antigen-presenting cell; CTL, cytolytic T lymphocyte; E:T, effector:target; NK, natural killer.

tains hemolytic and hemagglutinating activity, is as potent as live virus in inducing CD8+ antiinfluenza CTL responses (14).

Cytokines

IL-12 (specific activity 5×10^6 U/mg) was generously provided by Dr. Stanley Wolf (Immunology Department, Genetics Institute, Inc). Recombinant human IL-4, IL-15, and IL-7 were purchased from R & D Systems Inc. (Minneapolis, MN). ELISA kits measuring IL-12, IL-2, IL-6, TNFα, and IFNγ were purchased from R & D Systems, Inc.

Neutralizing antibodies

Polyclonal goat anti-human IL-12, IL-2R (alpha chain, CD25), and IFNγ antibodies were purchased from R & D Systems, Inc. The hybridoma producing neutralizing anti-human IL-12 monoclonal antibody (C8.6) was kindly provided by Dr. G. Trinchieri of the Wistar Institute (Philadelphia, PA).

Induction of CTL responses

Dendritic cells were infected with 1,000 HAU/ml (moi of 2–4) of different forms of live or heat-inactivated influenza virus for 1 h at 37°C in serum free medium, washed extensively, and added to bulk cultures of purified syngeneic T cells in 24-well or 48-well plastic dishes (Falcon Labware, Lincoln Park, NJ). After 7 d, the cells were harvested and distributed in varying numbers in 100-μl vol to 96-well microtiter plates. CTL activity was measured using a standard ^{51}Cr-release assay with infected or uninfected macrophages, as previously described (13). Effector:target (E:T) ratios were 30:1–40:1. In brief, macrophages were brought to 10^7/ml in serum-free medium, and infected with 1,000 HAU/ml of influenza virus. The cells were simultaneously labeled with ^{51}Cr by the addition of 400 μCi of $Na^{51}CrO_4$ (1 mCi/ml, sterile stock; New England Nuclear, Boston, MA) per milliliter. The targets were then washed four times, resuspended to 2×10^5/ml, and aliquoted in 50 μl vol to 96-well round bottom dishes containing effector cells. Percent specific ^{51}Cr release was calculated from the following formula: 100 × ([release by CTL − spontaneous release]/[total release − spontaneous release]). Spontaneous release was generally 15% of the total release.

Proliferation assays

2×10^5 bulk or CD8+ T cells were plated in 96-well flat bottomed plates (Costar, Cambridge, MA) in the presence or absence of varying concentrations of cytokines. APCs were partially purified dendritic cells (T:APC ratio of 5:1) or purified dendritic cells (T:APC ratio of 30:1). APCs were infected with influenza virus as described above. Uninfected APCs served as controls. Proliferation was determined on days 5–6 with the addition of 4 μCi/ml of ^{3}H-TdR for 12–16 h to triplicate microwells (mean cpm).

Results

IL-12 is not produced in dendritic cell-dependent CTL responses. Potent antiinfluenza CTL responses are induced from resting CD8+ T cells when dendritic cells are the APCs (13). Dendritic cells can produce IL-12 (15, 16) and direct the development of Th1 cells from CD4+ T cells (15). However, it is not known whether IL-12 is synthesized after infection with virus and during contact with CD8+ T cells. Using a sensitive ELISA system, we found that dendritic cells infected with live replicating influenza virus produced little to no IL-12 p70 heterodimer (range of 4–10 pg/ml in four separate experiments). Furthermore, significant levels of IL-12 were not detectable in T cell–dendritic cell culture supernatants over the 7-d period during which CD8+ CTLs normally develop (< 15 pg/ml). In contrast the cytokines IL-2, TNFα, IFNγ and IL-6 were detectable by ELISA (data not shown). Several other stimuli including LPS (reported to induce IL-12 mRNA in murine splenic dendritic cells, [17]), superantigens (staphylococcal enterotoxins), or addition of allogeneic T cells also failed to elicit high levels of IL-12 by dendritic cells.

In the CTL cultures, dendritic cells were added at 1 cell per 30 T cells. We considered the possibility that very low levels of IL-12 were produced in the supernatants. Neutralizing anti–IL-12 antibody was therefore added to the culture system. CTL responses were not blocked by anti–IL-12 antibody (5–25 μg/ml [Fig. 1] and data not shown) but were significantly suppressed by the addition of anti–IL-2 R antibody (α chain specific). IFNγ production was also unaffected by the addition of anti–IL-12 to the dendritic cell–T cell cultures. This suggests that endogenous IL-2, rather than IL-12, was responsible for IFNγ production. Indeed, IFNγ levels were markedly diminished by the addition of anti–IL-2 R α antibody (Fig. 1). Thus our data suggest that dendritic cells do not produce biologically significant levels of IL-12 after virus infection, and that IL-12 is not produced during the development of potent antiviral CTLs. The antiinfluenza CTLs that are generated in bulk T cell cultures by virus-infected dendritic cells are CD8+ (13). However, there is a substantial number of CD4+ T cells presumably responding to influenza antigens in a class II restricted fashion. Even their presence, however, was insufficient to elicit IL-12 from influenza infected dendritic cells.

IL-12 enhances T cell proliferative responses to influenza-virus infected dendritic cells. IL-12 stimulates the proliferation of activated T and NK cells but causes minimal proliferation of resting peripheral blood mononuclear cells (7). We evaluated the effects of IL-12 on enhancing T cell proliferative responses to influenza virus–infected dendritic cells. Marked increases in proliferative responses were noted with the addition of IL-12 at low doses (Fig. 2). IL-12 is known to enhance proliferative responses to mitogen-activated T cells in the picomolar range (7). This was true for both bulk T cells (Fig. 2 *A*),

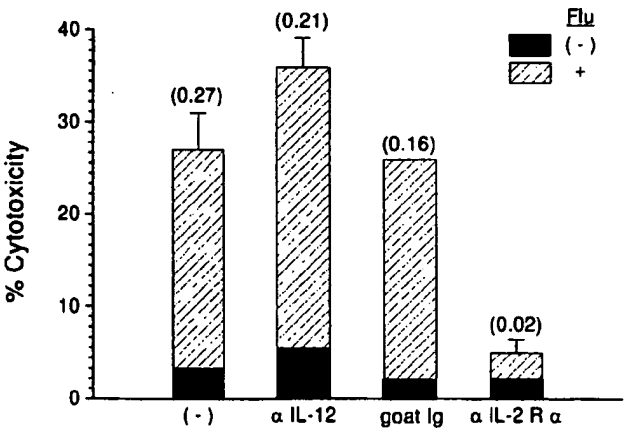


Figure 1. Antiviral CTL and IFNγ responses that are induced by dendritic cells are blocked by anti–IL-2 but not anti–IL-12 antibodies. Bulk T cells were cocultured with uninfected or influenza infected dendritic cells (T:DC ratio 30:1) in the presence of 5 μg/ml of goat anti–IL-12 ab and anti–IL-2R alpha chain ab, or goat IgG. After 7 d the effector cells were tested for cytolytic activity of influenza infected syngeneic macrophage targets in a Cr release assay. E:T ratio was 30:1. Lysis of uninfected macrophage targets was < 5% (data not shown). Similar results were obtained in three other experiments. The numbers in parentheses refer to the concentrations of IFNγ (ng/ml) in the supernatants on the third day of culture.

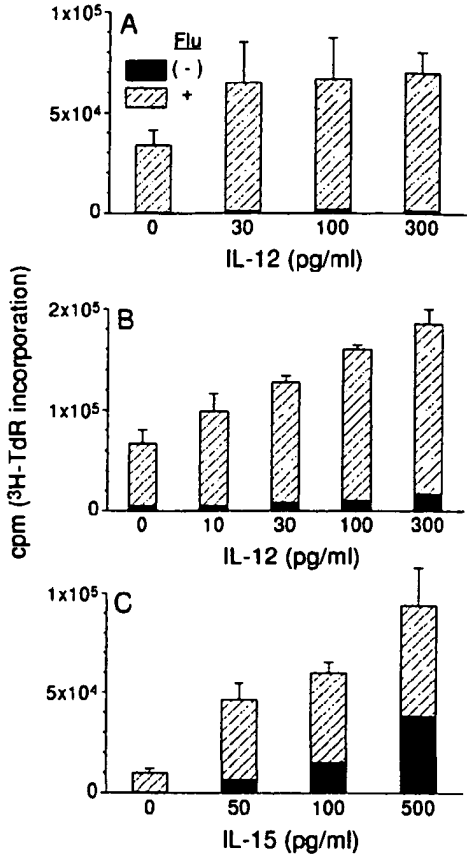


Figure 2. IL-12 enhances T cell proliferative responses to influenza virus-infected dendritic cells. Bulk T cells (*A*), (*C*) or CD8+ T cells (*B*), were cultured with virus-infected or uninfected APCs for 5 d. IL-12 (*A* and *B*) and IL-15 (*C*) were added in graded doses. Black portions of the bars represent the proliferative response to uninfected APCs while the hatched portion represent the responses to influenza-infected APCs. Proliferation was determined by the addition of ^{3}H-TdR for 16 h on day 5. Results are means of triplicates. The data are representative of two experiments.

as well as for highly purifed CD8+ T cells (Fig. 2 *B*). Background responses were minimal. T cell yields were increased up to twofold (data not shown). We also evaluated IL-15, a cytokine which shares many of the properties of IL-2 (18), and enhances CD4+ T cell-dependent responses to antigen (19). In contrast to IL-12, IL-15 produced dramatic background responses in bulk T cell populations (Fig. 2 *C*). Antigen specific T cell proliferative responses were still evident, however. IL-15 can induce some proliferation in bulk populations of human blood mononuclear cells (19). When IL-15 was added to purified CD8+ T cells, the responses generated to uninfected vs virus-infected dendritic cells was similar even with low doses, e.g., 50 pg/ml (data not shown).

IL-12 enhances weak antiviral cytolytic T cell responses. While dendritic cells induce strong CD8+ CTL responses to influenza virus, macrophages are weak or inactive. This may be due to apoptosis, observed 12–16 h after infection of macrophages with virus (20). However, macrophages are useful as target cells in short term CTL assays because > 70% get infected. B cells are poor stimulators of the T cell response to the virus, probably because they are infected poorly with influenza (13). As a result it is convenient for eliciting CTL responses to select macrophages as CTL targets, and to use T and macrophage-depleted blood populations as partially enriched sources of dendritic cells. The latter generally contain about 3–5% dendritic cells and are highly effective at inducing CD8+ CTL responses (13).

IL-12 (100 pg/ml) enhanced the cytolytic activity of bulk T cells towards influenza-infected, macrophage target cells (Fig. 3). Two situations were identified where IL-12 was most effective. When low doses of APCs were used (T:APC ratio = 30:1, Fig. 3A or T:dendritic cell ratio = 100:1, Fig. 3 *B*), IL-12 restored cytolytic activity to levels seen at higher doses of APCs. IL-12 was also effective when the blood donors had weak baseline antiinfluenza CTL responses (Fig. 3 *B*). The enhancing effects of IL-12 were also evident with highly purified CD8+ T cells that were stimulated with influenza-infected dendritic cells (Fig. 4). We confirmed that the responding cells in the preparation were primarily CD8+, by staining with monoclonal antibodies at the end of the 7-d culture period (data not shown).

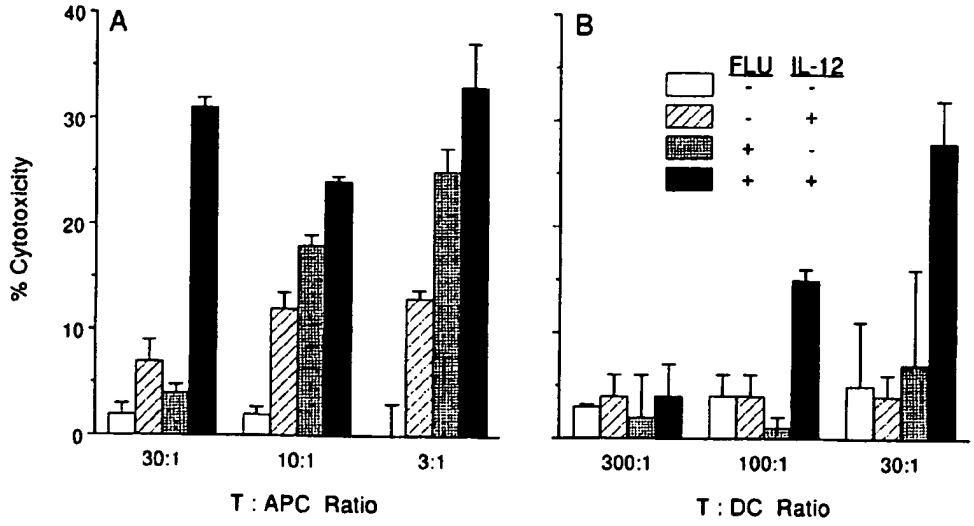


Figure 3. IL-12 enhances antiinfluenza virus CTL responses induced by dendritic cells. Partially enriched dendritic cells or "APCs" (*A*), or purified dendritic cells (*B*), were uninfected or infected with influenza virus and added at various ratios to bulk T cells. IL-12 was added at 100 pg/ml. Lytic activity was determined after 7 d on syngeneic macrophage-infected targets (E:T ratio = 40:1). Lysis of uninfected targets was < 5% (data not shown).

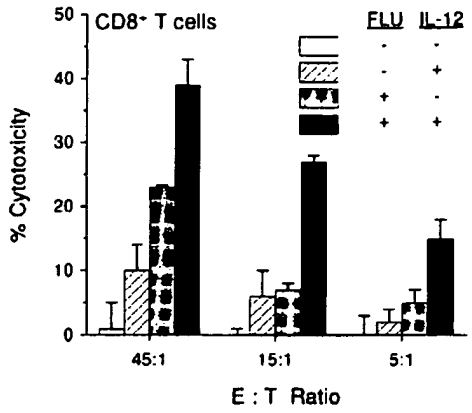


Figure 4. IL-12 enhances cytolytic T cell responses from purified CD8+ T cells. CD8+ T cells were cultured with uninfected or influenza virus-infected APCs (partially enriched dendritic cells) at T:APC ratio of 50:1 in the presence or absence of IL-12 (100 pg/ml). After 7 d, effector cells were harvested and lytic activity was determined on syngeneic macrophage-infected targets at various E:T ratios. Lysis of uninfected targets was < 5% at all E:T ratios used (data not shown). This result is typical of experiments with donors exhibiting CD8+ CTL responses of 20–30% without IL-12.

In summary, IL-12 appears to be most effective in enhancing CD8+ CTL responses when APC numbers are limiting or the CTL response of the donor is weak to begin with.

IFNγ is not responsible for the IL-12-induced enhancement of CTL responses. IL-12 has a major role in facilitating the production of IFNγ by peripheral blood lymphocytes (reviewed in [1]). IL-12 enhanced both antiviral cytolytic responses and IFNγ production in a dose-dependent manner (Fig. 5 *A*). The highest responses were observed at doses of 100–300 pg/ml, similar to that seen in the T cell proliferation assays (Fig. 2). Similar results were obtained when (*a*) CD8+ T cells were used (data not shown), or (*b*) heat inactivated,

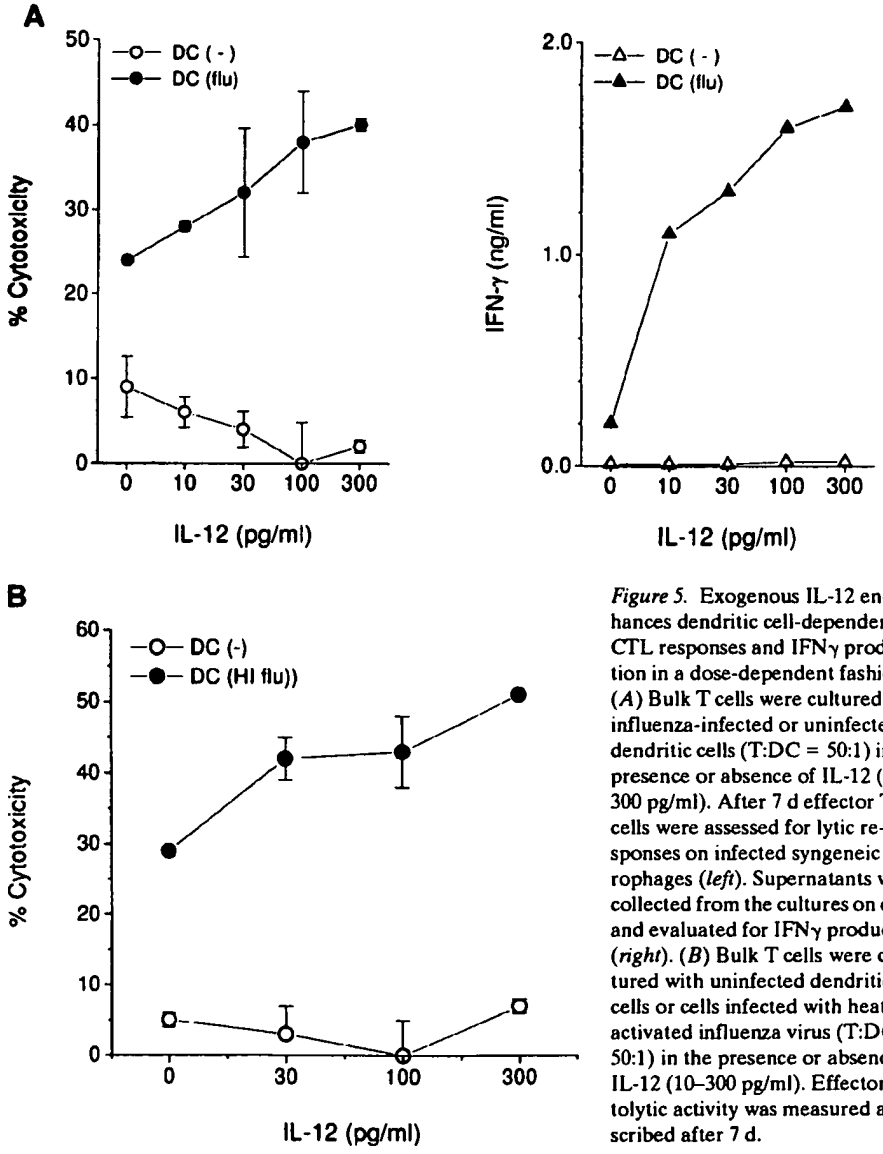


Figure 5. Exogenous IL-12 enhances dendritic cell-dependent CTL responses and IFNγ production in a dose-dependent fashion. (*A*) Bulk T cells were cultured with influenza-infected or uninfected dendritic cells (T:DC = 50:1) in the presence or absence of IL-12 (10–300 pg/ml). After 7 d effector T cells were assessed for lytic responses on infected syngeneic macrophages (*left*). Supernatants were collected from the cultures on day 3 and evaluated for IFNγ production (*right*). (*B*) Bulk T cells were cultured with uninfected dendritic cells or cells infected with heat inactivated influenza virus (T:DC = 50:1) in the presence or absence of IL-12 (10–300 pg/ml). Effector cytolytic activity was measured as described after 7 d.

Table I. Anti-IFNγ does not Block the CTL-enhancing Function of IL-12

HI-FLU	Treatment	Percentage specific lysis of targets MO [−]	MO [+]
−	goat Ig	0	5
−	anti-IFNγ	0	8
−	IL-12	0	5
−	IL-12 + goat Ig	0	5
−	IL-12 + anti-IFNγ	0	3
+	goat Ig	0	17
+	anti-IFNγ	0	17
+	IL-12	2	36
+	IL-12 + goat Ig	3	45
+	IL-12 + anti-IFNγ	3	34

Highly purified CD8+ T cells were cultured with uninfected dendritic cells or dendritic cells infected with heat-activated influenza virus (HI-FLU) in the presence or absence of interleukin 12 (300 pg/ml). The T:APC ratio was 50:1. Neutralizing goat anti-IFNγ or control goat IgG were added at a final concentration of 25 μg/ml on the first and fourth days of culture. Antiviral cytolytic activity was assessed on syngeneic uninfected, MO [−], or virus-infected macrophage targets, MO [+], after 7 days. The data are representative of three experiments.

replication deficient virus was used (Fig. 5 *B*). Even at low doses (10 pg/ml), there was greater than fivefold increase in the quantity of IFNγ produced. This sensitivity to IL-12 has been described in other culture systems (1, 5, 6).

We considered the possibility that IFNγ production induced by IL-12 was mediating the induction of CD8+ CTLs by virus-infected dendritic cells. Endogenous IL-12 production is critical to the generation of NK cell and T cell responses through the production of IFNγ, best demonstrated in models of bacterial and parasitic infections (reviewed in [1]). We added large doses of neutralizing anti-IFNγ antibody to cocultures of T cells and virus-infected dendritic cells in the presence of IL-12. As seen in Table I, the antibody did not block the induction of cytolytic activity in highly purified CD8+ T cell populations. We confirmed that significant amounts of IFNγ were being synthesized in the culture medium in response to IL-12 (up to 2.7 ng/ml). Since our cultures were extensively depleted of NK cells, it is unlikely that they were the source of IFNγ production (see below). We verified that the major responding cells in the cultures after 7 d were CD8+ T cells and not NK cells by FACS® analysis.

We determined that IL-12 was responsible for the majority of IFNγ production by CD8+ CTLs. Anti–IL-12 neutralizing antibody blocked the induction of IFNγ release induced by IL-12, as well as the enhanced CTL responses. Anti–IL-2 Rα only partially reduced the IFNγ release and CTL responses induced by IL-12 (by ~ 40%, data not shown), indicating that the majority of IFNγ produced is due to IL-12.

Comparison of a panel of cytokines on the enhancement of antiviral CTL responses. IL-12 was compared with a panel of cytokines that are also known to promote CD8+ CTL responses. IL-12 was the most potent enhancer of antigen-dependent CTLs (Fig. 6, *A* and *B*). IL-7, a growth factor implicated in the development of CD8+ CTLs (21), appeared to be considerably less effective even when added at 1 ng/mL Interestingly, IL-4, a key cytokine for the induction of Th2 responses, and which normally counteracts the effects of IL-12, also enhanced the CTL responses significantly. IL-15 increased the CTL response as well, but also caused a substantial rise in the background (Fig. 6, *hatched bars*), as noted previously in the T cell proliferation assays (Fig. 2). IL-2 was similar to IL-15 in inducing backgrounds and combinations of IL-2 and IL-12 were not synergistic in enhancing CTL responses (data not shown).

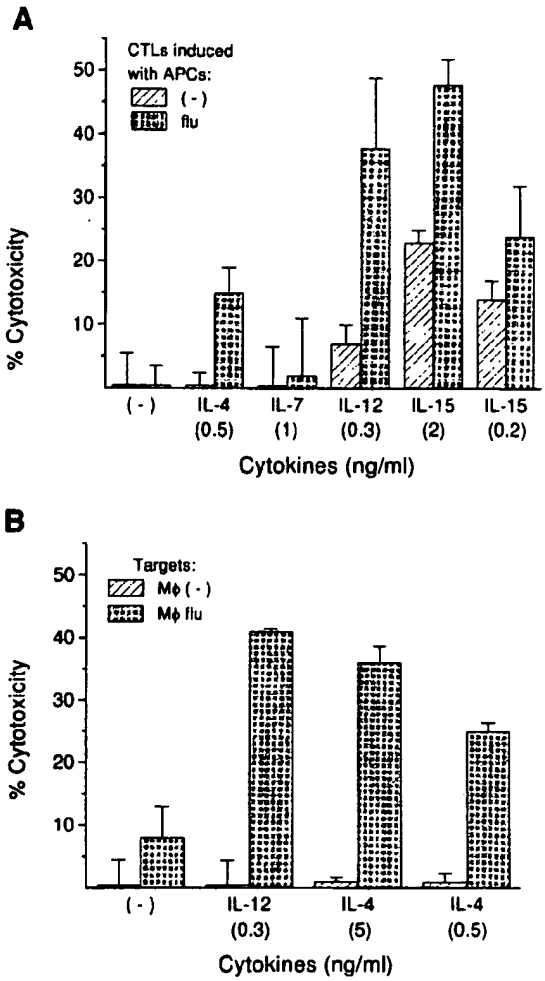


Figure 6. Both IL-12 and IL-4 can selectively enhance virus-specific CTL responses to influenza virus–infected dendritic cells. (*A*) Bulk T cells were stimulated with uninfected or influenza infected, partially purified dendritic cells (T:APC ratio of 5:1) for 7 d in the presence of various cytokines. CTL responses were tested on syngeneic influenza–infected macrophages. Note the high background with IL-15. (*B*) Bulk T cells were stimulated with purified dendritic cells at a T:DC ratio of 60:1. After 7 d effector cells were tested for virus specific CTL activity. Lysis of uninfected targets was < 5% at all E:T ratios used (data not shown).

Discussion

Activation of antiviral CD8+ CTLs by dendritic cells is enhanced by IL-12. Dendritic cells are specialized antigen-presenting cells for the induction of T cell–mediated immune responses (reviewed in [22]). Their efficacy is based on their ability to prime CD4+ and CD8+ T cells in small numbers and high ex-

pression of adhesins, costimulators, and intracellular vesicles critical for antigen presentation. Another means by which dendritic cells induce potent T cell responses is via the release of IL-12. Using murine TCR transgenic CD4+ T cells, Macatonia et al. (15) showed that dendritic cells induce the differentiation of naive T cells into IFNγ producing Th1 cells by synthesizing IL-12.

Dendritic cells induce potent human antiviral CD8+ CTL responses without the requirement for CD4+ T cells or exogenous cytokines (13). We hypothesized, therefore, that dendritic cells produce IL-12 when stimulating strong antiviral CD8+ CTL responses. However, dendritic cells did not produce significant levels of IL-12 after influenza virus infection or when stimulating either bulk or purified CD8+ T cells. Several stimuli known to produce IL-12 from human macrophages, e.g., LPS, *Staphylococcal aureus* Cowan strain 1, allogeneic T cells, in our hands induced little to no IL-12 when added to human blood dendritic cells. IL-12 may only be mobilized by dendritic cells after certain conditions of T cell activation (15). For example, interaction between CD40L on activated T cells and CD40 on APCs is critical for the priming of Th1 cells (23, 24). The CD8+ CTLs generated in our system may express only low levels of CD40L. Alternatively, the production of IL-12 may critically depend on the source and/or the maturational state of dendritic cells.

The addition of low doses of IL-12 to purified bulk or CD8+ T cells and influenza virus–infected dendritic cells increased both the proliferative and CTL activities substantially (2–20-fold). Our study provides the first evidence of the enhancing effects of IL-12 on human antiviral CD8+ T cell responses. In those instances where donors had weak or poor baseline antiviral responses, the effect of adding IL-12 was dramatic. A strong antiviral immune response was elicited where little to none could be previously detected (Figs. 3 and 6). We feel this is an amplification of a low level of CD8+ T cell memory rather than a primary response, since one would not expect to detect levels of 30–40% CTL lysis in a primary response. While enhancement to such levels are seen with IL-12 in CTL responses to alloantigens or after stimulation with anti-CD3, there the number of responsive T cells is very high (9, 10, 12, 25). IL-12 was also effective when the dose of dendritic cells was less than optimal or limiting (Fig. 3).

IL-12 directly amplifies the antiviral effects of highly purified CD8+ T cells. The amplification of both the proliferative and the antiviral CTL responses correlated with the amount of IFNγ produced in the cultures. In these respects, IL-12 effects on enhancing antiviral CTLs is like the effects on bulk NK cells (5, 6), as long as antigen is being presented by dendritic cells.

Mechanism of IL-12 in enhancing CTL responses. IL-12 likely increases CTL responses through several ways. By functioning as a growth factor (7), it increases the number of T cells responding to antigen (up to twofold as shown here and reported in reference 12), and enhances the production of IL-2 (19, 26) and/or the expression of the IL-2 high affinity receptor (CD25, [27]). The effect of IL-12 may be direct since it is not blocked by anti–IL-2Rα (7, 10–12). Activation of NK cells by IL-12 is similarly not dependent on cytokines such as IL-2 (1, 5, 6). In our system IL-2 was critical, since the addition of anti–IL-2Rα antibody substantially blocked CTL responses as described by others (9).

IL-12 may also upregulate the expression of perforin (11), serine esterase levels and cytotoxic granules in CD8+ CTLs (10, 12). A direct increase in the the CTL precursor frequency has also been postulated. However, we were unable to measure enhanced CTLp levels under limiting dilution conditions (data not shown).

IL-12–induced IFNγ could directly enhance antiviral responses through its effects on infected target cells. This is one mechanism by which NK cells clear virus (28). We found, however, that the addition of neutralizing anti-IFNγ antibodies did not block either the induction of or killing capacity of CD8+ CTLs in the presence of IL-12. These findings are in concordance with those of Mehrortra et al. (12) who showed that the synergistic effects of IL-2 and IL-12 on CTL generation were not blocked by anti-IFNγ, and Bloom et al. (11) in the murine system where allospecific CTL generation was studied.

Our results do not rule out a role for IFNγ in antiinfluenza responses, however, or of cytokines such as TNFα which are also produced in response to IL-12. This is because our assays are designed to measure cytolytic activity of virus-infected target cells vs elimination of virus through other inhibitory mechanisms. IFNγ can clear infection by affecting viral RNA synthesis and stability, replication, and induction of mediators such as nitric oxide (29). Such mechanisms, rather than direct cytolytic activity, are thought to be the primary means by which hepatitis virus and other cytopathic infections are cleared (30).

When compared to other T cell growth factors, IL-12 appeared to be the most efficient at enhancing CTL responses. IL-2 and IL-15, while partly effective, gave high background responses possibly because of effects on IL-2 Rβ expressing T cells. IL-15 was tested because it can amplify dendritic cell-dependent T cell responses (31) and increases antigen-specific proliferative T cell responses (19). IL-12 and IL-15 are synergistic in augmenting NK cell CTL function and IFNγ production (18), but were not evaluated together because of the observed background activity of IL-15. Besides inducing the development of Th2 cells from naive CD4+ T cells, IL-4 can augment CTL responses including antiinfluenza CTL responses (32), as shown here (Fig. 6). The pathway by which IL-4 enhances dendritic cell-dependent CTL responses is not known. However, IL-4 is known to prime PBMC for IL-12 production in response to LPS or *S. aureus* (33). It is possible that the effects of IL-4 seen here are mediated through dendritic cells that have been primed for IL-12 production. IL-7, which has been used with dendritic cells to induce CTL responses (21) was not effective in our studies.

Restricted requirements for IL-12 in antiviral responses. The regulatory influence of IL-12 on cellular immune responses is most striking in animal models of bacterial and protozoal infection (reviewed in references 1 and 2). Surprisingly, less is known about the role of IL-12 in T cell–mediated resistance to viral infections. For instance, little is known about whether IL-12 plays a role in the development of antiviral CD8+ CTLs.

In two animal models of virus infection, lactate dehydrogenase elevating virus (34) and MCM virus infections (28), either message for IL-12 or protein is detected in macrophages and splenic cells shortly after infection. However, the primary mode of virus clearance, at least in MCMV infection, appears to be via NK cells through secretion of IFNγ, rather than CD8+ T cells. This antiviral pathway is blocked with anti–IL-12 antibody or enhanced with administration of low dose IL-12 (28). In contrast, CD8+ T cell expansion is unaffected by IL-12 neutralizing antibody. In other virus infections IL-12 is not readily detectable, e.g., LCMV infection, and viral clear-

ance is primarily dependent on the activation of CD8+ CTLs. While low doses of IL-12 enhance splenic CD8+ T cell numbers in LCMV- infected mice and decrease viral replication, cytolytic activity is not increased (35). Furthermore, the administration of anti-IL-12 antibody has no demonstrable effect on late T cell responses (28). Thus we would predict that the ability to rapidly induce significant IL-12 levels in vivo will determine whether NK cells are major contributors to antiviral defense.

In humans, there is evidence to suggest that IL-12 has a critical function in the progression of HIV infection (26). PBMCs from patients infected with this virus are deficient in their production of IL-12, and have suboptimal Th1 immune responses (36). Addition of IL-12 to T cells in vitro, restores recall responses to antigens (19, 26). It is presumed, but not established, that IL-12 deficiency also affects antiviral CD8+ T cell development and function.

In the case of influenza virus, significant levels of IL-12 were not produced by either macrophages or dendritic cells after infection in vitro. IL-12 is reportedly produced in the lungs of mice as early as day 3 after infection with influenza virus, but it is not known which cells synthesize the cytokine (37). Neutralizing anti–IL-12 antibodies increase morbidity in virus-infected mice compared with uninjected animals, suggesting that the cytokine is essential for a potent antiviral immune response (28). Influenza virus infection is known to elicit IFNγ production in infected human PBMCs (38). Perhaps CD40L-bearing helper T cells are major inducers of IL-12 production by dendritic cells in infected lungs. Whether IL-12 is a requisite in vivo for clearance of influenza virus in humans cannot be determined from these experiments. Optimal clearing of influenza virus after infection likely requires CD8+ CTL effector activity (39).

Applications. IL-12 may be a useful immunomodulator when used in conjunction with dendritic cells, to enhance antigen-specific class I restricted CD8+ CTL responses in viral infections and tumors. Peptide pulsed dendritic cells induce protective immune responses and also cause tumor regression of established metastases (40–42). The concomitant use of IL-12 may reduce the need for large numbers or doses of dendritic cells. Similarly, by using dendritic cells, it may be feasible to use very low doses of IL-12. Large doses of IL-12 have exhibited toxicities in both humans and in animals (43). In HIV infection, PBMC from infected patients demonstrate impaired IL-12 production (36). Immunotherapy may be most effective in later stages of the disease when CD4+ T cell counts fall and CD8+ CTL function declines. Here, IL-12 could concomitantly enhance virus-specific CTL responses as well as CD4+ helper cell responses, the latter as previously described (26).

Acknowledgments

We thank Dr. R.M. Steinman for advice and review of the manuscript, Dr. S. Wolf for recombinant human IL-12, Dr. G. Trinchieri for anti–IL-12 antibodies, and Judy Adams for graphics.

These studies were supported by grants from the National Institutes of Health (AR-39552, AR-42557 to N. Bhardwaj), and the Irma T. Hirschl and the New York Community Trusts (N. Bhardwaj).

References

1. Trinchieri, G. 1995. Interleukin-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. *Annu. Rev. Immunol.* 13:251–276.

2. Trinchieri, G. 1994. Interleukin-12: a cytokine produced by antigen-presenting cells with immunoregulatory functions in the generation of T-helper cells type 1 and cytotoxic lymphocytes. *Blood.* 84:4008–4027.

3. Scharton, T.M., and P. Scott. 1993. Natural killer cells are a source of interferon gamma that drives differentiation of CD4+ T cell subsets and induces early resistance to Leishmania major in mice. *J. Exp. Med.* 178:567–577.

4. Chehimi, J., N.M. Valiante, A. D'Andrea, M. Rengaraju, Z. Rosado, M. Koyayashi, B. Perussia, S.F. Wolf, S.E. Starr, and G. Trinchieri. 1993. Enhancing effect of natural killer cell stimulatory factor (NKSF/interleukin-12) on cell-mediated cytotoxicity against tumor-derived and virus-infected cells. *Eur. J. Immunol.* 23:1826–1830.

5. Chan, S.H., B. Perussia, J.W. Gupta, M. Kobayashi, M. Pospisil, H.A. Young, S.F. Wolf, D. Young, S.C. Clark, and G. Trinchieri. 1991. Induction of interferon γ production by natural killer cell stimulatory factor: characterization of the responder cells and synergy with other inducers. *J. Exp. Med.* 173: 869–879.

6. Kobayashi, M., L. Fitz, M. Ryan, R.M. Hewick, S.C. Clark, S. Chan, R. Loudon, F. Sherman, B. Perussia, and G. Trinchieri. 1989. Identification and purification of natural killer cell stimulatory factor (NKSF), a cytokine with multiple biologic effects on human lymphocytes. *J. Exp. Med.* 170:827–845.

7. Gately, M.K., B.B. Desai, A.G. Wolitzky, P.M. Quinn, C.M. Dwyer, F.J. Podlaski, P.C. Familletti, F. Sinigaglia, R. Chizonnite, U. Gubler, and A.S. Stern. 1991. Regulation of human lymphocyte proliferation by a heterodimeric cytokine, IL-12 (cytotoxic lymphocyte maturation factor). *J. Immunol.* 147:874–882.

8. Seder, R.A., and W.E. Paul. 1994. Acquisition of lymphokine-producing phenotype by CD4+ T cells. *Annu. Rev. Immunol.* 12:635–673.

9. Gately, M.K., A.G. Wolitzky, P.M. Quinn, and R. Chizzonite. 1992. Regulation of human cytolytic lymphocyte responses by interleukin-12. *Cell. Immunol.* 143:127–142.

10. Chouaib, S., J. Chehimi, L. Bani, N. Genetet, T. Tursz, F. Gay, G. Trinchieri, and F. Mami-Chouaib. 1994. Interleukin 12 induces the differentiation of major histocompatibility complex class I-primed cytotoxic T-lymphocyte precurosrs into allospecific cytotoxic effectors. *Proc. Natl. Acad. Sci. USA.* 91: 12659–12663.

11. Bloom, E.T., and J.A. Horvath. 1994. Cellular and molecular mechanisms of the IL-12-induced increase in allospecific murine cytolytic T cell activity. Implications for the age-related decline in CTL. *J. Immunol.* 152:4242–4254.

12. Mehrotra, P.T., D. Wu, J.A. Crim, H.S. Mostowski, and J.P. Siegel. 1993. Effects of IL-12 on the generation of cytotoxic activity in human CD8+ T lymphocytes. *J. Immunol.* 151:2444–2452.

13. Bhardwaj, N., A. Bender, N. Gonzalez, L.K. Bui, M.C. Garrett, and R.M. Steinman. 1994. Influenza virus-infected dendritic cells stimulate strong proliferative and cytolytic responses from human CD8+ T cells. *J. Clin. Invest.* 94:797–807.

14. Bender, A., L.K. Bui, M.A.V. Feldman, M. Larsson, and N. Bhardwaj. 1995. Inactivated influenza virus, when presented on dendritic cells, elicits human CD8+ cytolytic T cell responses. *J. Exp. Med.* 182:1663–1671.

15. Macatonia, S.E., N.A. Hosken, M. Litton, P. Vieira, C. Hsieh, J.A. Culpepper, M. Wysocka, G. Trinchieri, K.M. Murphy, and A. O'Garra. 1995. Dendritic cells produce IL-12 and direct the development of Th1 cells from naive CD4+ T cells. *J. Immunol.* 154:5071–5079.

16. Kang, K.F., M. Kubin, K.D. Cooper, S.R. Lessin, G. Trinchieri, and A.H. Rook. 1996. IL-12 synthesis by human Langerhans cells. *J. Immunol.* 156: 1402.

17. Kanangat, S., S. Nair, J.S. Babu, and B.T. Rouse. 1995. Expression of cytokine mRNA in murine splenic dendritic cells and better induction of T cell-derived cytokines by dendritic cells than by macrophages during in vitro costimulation assay using specific antigens. *J. Leukocyte Biol.* 57:310–316.

18. Carson, W.E., J.G. Giri, M.J. Lindemann, M.L. Linett, M. Ahdieh, R. Paxton, D. Anderson, J. Eisenmann, K. Grabstein, and M.A. Caligiuri. 1994. Interleukin (IL) 15 is a novel cytokine that activates human natural killer cells via components of the IL-2 receptor. *J. Exp. Med.* 180:1395–1403.

19. Seder, R.A., K.H. Grabstein, J.A. Berzofsky, and J.F. McDyer. 1995. Cytokine interactions in human immunodeficiency virus-infected individuals: roles of interleukin IL-2, IL-12, and IL-15. *J. Exp. Med.* 182:1067–1078.

20. Fesq, H., M. Bacher, M. Nain, and D. Gemsa. 1994. Programmed cell death (apoptosis) in human monocytes infected by influenza virus. *Immunobiology* 190:175–182.

21. Celis, E., V. Tsai, C. Crimi, R. DeMars, P.A. Wentworth, R.W. Chestnut, H.M. Grey, A. Sette, and H.M. Serra. 1994. Induction of anti-tumor cytotoxic T lymphocytes in normal humans using primary cultures and synthetic peptide epitopes. *Proc. Natl. Acad. Sci. USA.* 91:2105–2109.

22. Steinman, R.M. 1991. The dendritic cell system and its role in immunogenicity. *Annu. Rev. Immunol.* 9:271–296.

23. Shu, D., M. Kiniwa, C.Y. Wu, C. Maliszewski, N. Vezzio, J. Hakimi, M. Gately, and G. Delespesse. 1995. Activated T cells induce interleukin-12 production by monocytes via CD40-CD40 ligand interaction. *Eur. J. Immunol.* 25: 1125–1128.

24. Stuber, E., W. Strober, and M. Neurath. 1996. Blocking the CD40L-CD40 interaction in vivo specifically prevents the priming of T helper 1 cells through the inhibition of interleukin 12 secretion. *J. Exp. Med.* 183:693–698.

25. Young, J.W., and R.M. Steinman. 1990. Dendritic cells stimulate primary human cytolytic lymphocyte responses in the absence of CD4+ helper T cells. *J. Exp. Med.* 171:1315–1332.

26. Clerici, M., D.R. Lucey, J.A. Berzoksky, L.A. Pinto, T.A. Wynn, S.P. Blatt, M.J. Dolan, C.W. Hendrix, S.F. Wolf, and G.M. Shearer. 1993. Restoration of HIV-specific cell-mediated immune responses by interleukin-12 in vitro. *Science (Wash. DC).* 262:1721–1724.

27. Robertson, M.J., R.J. Soiffer, S.F. Wolf, T.J. Manley, C. Donahue, D. Young, S.H. Herrmann, and J. Ritz. 1992. Response of human natural killer (NK) cells to NK cell stimulatory factor (NKSF): cytolytic activity and proliferation of NK cells are differentially regulated by NKSF. *J. Exp. Med.* 175:779–788.

28. Orange, J.S., and C.A. Biron. 1996. An absolute and restricted requirement for IL-12 in natural killer cell IFN-gamma production and antiviral defense. *J. Immunol.* 156:1138–1142.

29. Muller, U., U. Steinhoff, L.F.L. Reis, S. Hemmi, J. Pavlovic, R.M. Zinkernagel, and M. Aguet. 1994. Functional role of type I and type II interferons in antiviral defense. *Science (Wash. DC).* 264:1918–1921.

30. Guidotti, L.G., T. Ishikawa, M.V. Hobbs, B. Matzke, R. Schreiber, and F.V. Chisari. 1996. Intracellular inactivation of the hepatitis B virus by cytotoxic T lymphocytes. *Immunology.* 4:25–36.

31. Jonuleit, H., U. Kuhn, G. Muller, M. Wolfl, S. Lohmann, J. Saloga, D. Becker, J. Knop, and A.H. Enk. 1995. Keratinocyte-derived IL-15 enhances accessory functions of epidermal LC and blood dendritic cells. *J. Invest. Dermatol.* 105:861 (Abstr.).

32. Horohov, D.W., J.A. Crim, P.L. Smith, and J.P. Siegel. 1988. IL-4 (B cell-stimulatory factor 1) regulates multiple aspects of influenza virus-specific cell-mediated immunity. *J. Immunol.* 141:4217–4224.

33. D'Andrea, A., X. Ma, M. Aste-Amezaga, C. Paganin, and G. Trinchieri. 1995. Stimulatory and inhibitory effects of IL-4 and IL-13 on the production of cytokines by human peripheral blood mononuclear cells: priming for IL-12 and tumor necrosis factor alpha production. *J. Exp. Med.* 181:537–546.

34. Coutelier, J.-P., J. Van Broeck, and S.F. Wolf. 1995. Interleukin-12 gene expression after viral infection in the mouse. *J. Virol.* 69:1955–1958.

35. Orange, J.S., S.F. Wolf, and C.A. Biron. 1994. Effects of IL-12 on the response and susceptibility to experimental viral infections. *J. Immunol.* 152:1253.

36. Chehimi, J., S.E. Starr, I. Frank, A. D'Andrea, X. Ma, R.R. Macgregor, J. Sennelier, and G. Trinchieri. 1994. Impaired interleukin-12 production in human immunodeficiency virus-infected patients. *J. Exp. Med.* 179:1361–1366.

37. Monteiro, J.M., and G. Trinchieri. 1995. Role of IL-12 in the in vivo immune response to influenza infection. *In* Interleukin 12: Cellular and Molecular Immunology of an Important Regulatory Cytokine, New York Acad. Sci. Conf. P24. (Abstr.)

38. Yamada, Y.K., A. Meager, A. Yamada, and F.A. Ennis. 1986. Human interferon alpha and gamma production by lymphocytes during the generation of influenza virus-specific cytotoxic T lymphocytes. *J. Gen. Virol.* 67:2325–2334.

39. McMichael, A. 1994. Cytotoxic T lymphocytes specific for influenza virus. *Curr. Top. Microbiol. Immunol.* 189:75–91.

40. Paglia, P., C. Chiodoni, M. Rodolfo, and M.P. Colombo. 1996. Murine dendritic cells loaded in vitro with soluble protein prime CTL against tumor antigen in vivo. *J. Exp. Med.* 183:317–322.

41. Mayordomo, J.I., T. Zorina, W.J. Storkus, L. Zitvogel, C. Celluzzi, L.D. Falo, C.J. Melief, S.T. Ilstad, W.M. Kast, A.B. DeLeo, and M.T. Lotze. 1995. Bone marrow-derived dendritic cells pulsed with synthetic tumour peptides elicit protective and therapeutic antitumour immunity. *Nature Med.* 1:1297–1302.

42. Celluzzi, C.M., J.I. Mayordomo, W.J. Storkus, M.T. Lotze, and L.D. Falo, Jr. 1996. Peptide-pulsed dendritic cells induce antigen-specific, CTL-mediated protective tumor immunity. *J. Exp. Med.* 183:283–287.

43. Orange, J.S., T.P. Salazar-Mather, S.M. Opal, R.L. Spencer, A.H. Miller, B.S. McEwen, and C.A. Biron. 1995. Mechanism of interleukin 12-mediated toxicities during experimental viral infections: role of tumor necrosis factor and glucocorticoids. *J. Exp. Med.* 181:901–914.

Rapamycin inhibits macropinocytosis and mannose receptor–mediated endocytosis by bone marrow–derived dendritic cells

Holger Hackstein, Timucin Taner, Alison J. Logar, and Angus W. Thomson

Dendritic cells (DCs) are professional antigen-presenting cells (APCs) that use 2 major pathways for antigen uptake: constitutive macropinocytosis and mannose receptor–mediated endocytosis. Efficient endocytosis is critical for DCs to fulfill their sentinel function in immunity. We investigated the influence of the immunosuppressive macrolide rapamycin on macropinocytosis of fluorescein isothiocyanate (FITC)–albumin and mannose receptor–mediated endocytosis of FITC-dextran by murine bone marrow–derived DCs by flow cytometry. The data show that (1) at a low, physiologically relevant concentration (1 ng/mL), rapamycin impairs macropinocytosis and mannose receptor–mediated endocytosis; (2) the effects are independent of DC maturation and can be demonstrated specifically in immature CD11c$^+$ major histocompatibility complex (MHC) class II^{lo} DCs by 3-color flow cytometry; (3) inhibition of endocytosis is not related to apoptotic cell death; and (4) molar excess of the structurally related molecule FK506 inhibits the actions of rapamycin. The inhibitory effects of rapamycin on DC endocytosis were confirmed in vivo. To our knowledge, this is the first report that a clinically relevant immunosuppressant inhibits DC endocytosis. (Blood. 2002;100:1084-1087)

Introduction

Dendritic cells (DCs) arise from CD34$^+$ bone marrow (BM) stem cells and represent a heterogenous population of ubiquitously distributed antigen-presenting cells (APCs) that play critical roles as initiators and modulators of immune responses.[1,2] Among the most striking features underlying the efficiency of DCs as APCs is their unsurpassed capacity to take up antigens via constitutive macropinocytosis and mannose receptor–mediated endocytosis[3] and to subsequently process and present major histocompatibility complex (MHC)–antigen complexes on their surface.[1] The capacity of DCs to endocytose and to present antigens is under tight developmental control: immature DCs are excellent at internalizing antigens but express low surface levels of MHC class II molecules, whereas mature DCs down-regulate endocytotic activity and up-regulate MHC class II and costimulatory molecules (CD40, CD80, CD86) that promote T-cell activation.[1] Anti-inflammatory drugs such as corticosteroids[4] or salicylates[5] suppress DC maturation and as a consequence enhance their endocytotic activity. Recent reports point toward Rho family proteins Cdc42[6] and Rac,[7] as well as aquaporins,[8] as important factors that regulate DC endocytosis. Rapamycin is a potent immunosuppressive macrolide, isolated from *Streptomyces hygroscopicus,* that inhibits downstream signaling from the targets of rapamycin proteins (TORs) by forming a complex with its intracellular receptor FK506-binding protein 12 (FKBP12) and TORs.[9] It is used clinically to prevent and treat allograft rejection.[10-12] Interaction of the rapamycin-FKBP12 complex with TORs results in inhibition of multiple biochemical pathways (eg, p70 S6 kinase, cyclin-dependent kinases, translational effector proteins) that are critical for cytokine/growth factor–induced cellular proliferation, ribosome biosynthesis, translation initiation, and cell cycle progression into S phase.[9,13] In view of the paucity of information concerning the influence of rapamycin on APC function and its well-documented inhibitory effects on protein synthesis, we analyzed the impact of rapamycin on DC endocytosis. Our results indicate that rapamycin is an inhibitor of DC endocytosis in vitro and in vivo and that molar excess of the structurally related immunophilin ligand FK506 partially reverses its inhibitory effects.

Study design

Generation of BM-derived DCs

Male C57BL/10J (I-A^b) mice, 8 to 12 weeks old, were purchased from the Jackson Laboratory (Bar Harbor, ME). BM-derived DCs were generated and characterized as described,[5] with minor modifications. Briefly, BM cells were cultured for 7 days in RPMI-1640 with 10% heat-inactivated fetal calf serum (FCS), L-glutamine, nonessential amino acids, sodium pyruvate, penicillin-streptomycin, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), 2-mercaptoethanol (2-ME) (all from Life Technologies, Gaithersburg, MD), 1000 U/mL murine granulocyte-macrophage colony-stimulating factor (GM-CSF; Schering-Plough, Kenilworth, NJ) ± 1000 U/mL murine interleukin 4 (IL-4) (R&D Systems, Minneapolis, MN). At day 2, 1-100 ng/mL rapamycin (Sigma, St Louis, MO) ± 20-250 ng/mL FK506 (Prograf for intravenous use, Fujisawa Healthcare, Deerfield, IL) was added. Every 2 days, 75% supernatant was

From the Thomas E. Starzl Transplantation Institute and Department of Surgery and the Department of Molecular Genetics and Biochemistry, University of Pittsburgh, PA, and the Institute of Clinical Immunology and Transfusion Medicine, Justus-Liebig University, Giessen, Germany.

Submitted October 29, 2001; accepted April 2, 2002.

Supported by the National Institutes of Health (grants R01 DK49745 and R01 AI41011 to A.W.T.). H.H. is supported by a scholarship from the Stiftung Hämotherapie-Forschung, Bonn, Germany.

H.H. and T.T. contributed equally to this work.

Reprints: Angus W. Thomson, Thomas E. Starzl Transplantation Institute, University of Pittsburgh, W1544 BST, 200 Lothrop St, Pittsburgh, PA 15213; e-mail: thomsonaw@msx.upmc.edu.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 U.S.C. section 1734.

replaced with fresh cytokine-containing medium (± rapamycin, FK506). On day 4, nonadherent cells were removed; on day 7, ≥ 50% of the nonadherent cells expressed CD11c.

In vivo administration of rapamycin

Rapamycin (Wyeth-Ayerst, Princeton, NJ) was dissolved in 51% wt/vol polyethylene glycol 300 (PEG300), 2.5% wt/vol polysorbate 80, 10% vol/vol ethanol (vehicle; reagents from Sigma). Chinese hamster ovary (CHO) cell-derived rhuman Flt3 ligand (10μg/kg/d, intraperitoneally; Immunex, Seattle, WA) was used to expand DCs in mice over a period of 10 days.[14] Animals were injected with rapamycin (0.5 mg/kg/d, intraperitoneally) or vehicle at the same times. Spleens were disaggregated with fine scissors and passed through a nylon cell strainer (Falcon Becton Dickinson, NJ) to obtain a single-cell suspension. Erythrocytes were lysed with 0.83% wt/vol NH_4Cl.

Endocytosis

Quantitative analysis of endocytosis was performed as described,[5] with minor modifications. Cells (5×10^5) were incubated with 5 μg/mL FITC-albumin (Sigma) or 0.1 mg/mL FITC-dextran (MW 42000, Sigma) at either 37°C or 4°C for 60 minutes (for in vivo–expanded DCs, 500 μg/mL FITC-albumin and 1 mg/mL FITC-dextran for 40 minutes). Endocytosis was stopped by 2 washes in ice-cold 0.1% sodium azide/1% FCS/phosphate-buffered saline (PBS). Cells were stained for CD11c (HL3) and, in some experiments, for MHC class II expression (IA^b β-chain, 25-9-17) as described (monoclonal antibodies from BD PharMingen, San Diego, CA).[5]

Apoptotic cell death

Apoptosis was analyzed by staining of phosphatidylserine translocation with FITC–annexin-V in combination with the vital dye 7-AAD (BD PharMingen) and detection of DNA fragmentation by terminal deoxynucleotidyl transferase–mediated fluorescein–2′-deoxyuridine 5′-triphosphate (dUTP) labeling (TUNEL assay; Roche Molecular Biochemicals, Indianapolis, IN) according to the manufacturer's instructions. Positive controls included deoxyribonuclease (DNase) treatment and ultraviolet C (UVC) irradiation (60 J/m²) in the TUNEL and annexin-V assay, respectively. Cells were costained for CD11c expression and analyzed by means of an EPICS XL flow cytometer (Beckman Coulter, Hialeah, FL).

Statistics

Statistical analysis was performed with a 2-tailed Student *t* test. Normal distribution of values was proved by the Kolomogorov-Smirnov test.

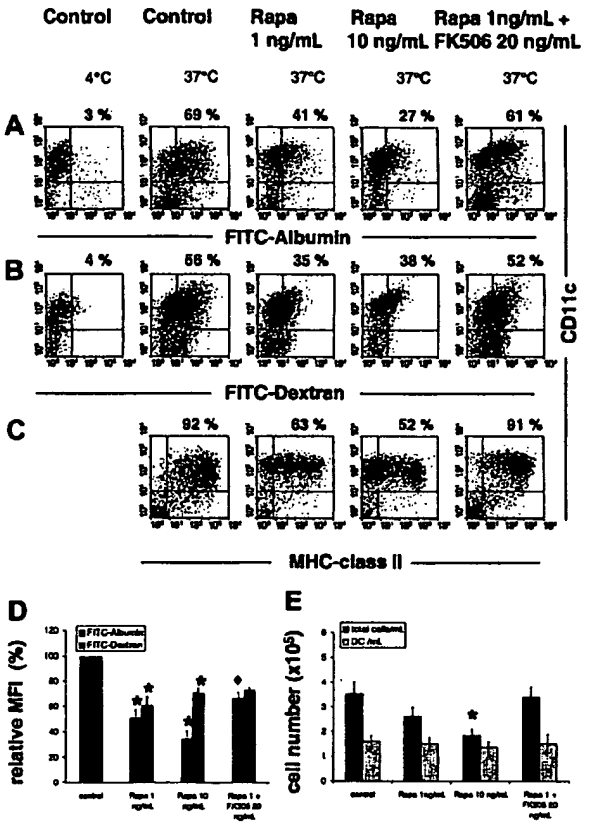


Figure 1. Rapamycin inhibits endocytosis by GM-CSF+IL-4–expanded DC. (A-D) BM-derived DCs were expanded for 7 days as described in "Study design"; rapamycin (Rapa) was added at day 2 (± FK506) at the concentrations indicated. FITC-albumin and FITC-dextran internalization at 37°C and 4°C (negative control), MHC class II (IA^b β-chain), and CD11c expression were analyzed by flow cytometry. In all experiments, $CD11c^+$ cells were analyzed to determine endocytosis and MHC class II expression specifically in DCs. (A-C) Numbers indicate the percentage of $CD11c^+$ cells that were positive for the marker indicated. (D) Data represent mean values (± SE) of $CD11c^+$ cells after subtraction of background fluorescence (4°C). (E) Comparison of total cell and DC numbers on day 7 of culture. (D, E) Differences between paired cultures were compared by 2-tailed Student *t* test for paired samples (*$P < .05$ vs control; ♦ $P < .05$ vs Rapa 1). (A-D) Results are representative of 4 (FITC-dextran) and 6 (FITC-albumin) separate experiments. (E) Results are representative of 6 separate experiments.

Results and discussion

Analysis of GM-CSF+IL-4–expanded, BM-derived DCs harvested at day 7 of culture revealed a reduced capacity of cells exposed to rapamycin to exhibit macropinocytosis of FITC-albumin and mannose receptor–mediated endocytosis of FITC-dextran. This was evident with respect to both the incidence (Figure 1A, B) and the mean fluorescence intensity (MFI; Figure 1D) of $CD11c^+$ cells. CD11c is a very reliable marker for murine DCs[15] and is not expressed in significant amounts by murine macrophages.[5,16] Because DC maturation is associated with marked down-regulation of endocytotic capacity,[3] we questioned whether these effects might be related to a stimulatory effect of rapamycin on DC maturation. However, rapamycin-exposed $CD11c^+$ DCs displayed significantly decreased surface MHC class II (Figure 1C) and costimulatory molecules (data not shown), indicating that they were more immature than untreated, control cells. It could be argued that the analysis of a fixed number of DCs by fluorescence-activated cell sorting (FACS) might mask an inhibitory effect of rapamycin on the differentiation of precursor cells into DCs. As shown in Figure 1E, however, rapamycin inhibited *total cell expansion* in a dose-dependent manner (antagonizable by a molar excess of FK506) but did not significantly block the *differentiation* of precursor cells into $CD11c^+$ DCs, as evidenced by the comparable yield of $CD11c^+$ DCs. Achievement of similar DC yields was due to consistently increased percentages of $CD11c^+$ DCs with characteristic DC morphology in the rapamycin-treated cultures.

Because additional experiments indicated that the inhibitory effect of rapamycin on DC maturation was IL-4 dependent (H.H., T.T., unpublished observations, October 2001), we expanded DCs with GM-CSF only. Generation of murine DCs in GM-CSF is a well-established culture method.[17] Additionally, to determine more precisely the endocytotic activity of homogenous DCs at the same stage of maturation, we specifically analyzed immature MHC II^{lo} DCs. These experiments confirmed the inhibitory effects of rapamycin on DC endocytosis and indicated that they were not IL-4 related (Figure 2A-C). Again, low concentrations of 1 ng/mL rapamycin (1.1 nmol) were sufficient to significantly and markedly suppress endocytotic activity. When 1 ng/mL rapamycin was used, the relative MFI of immature MHC class II^{lo} $CD11c^+$ DCs compared

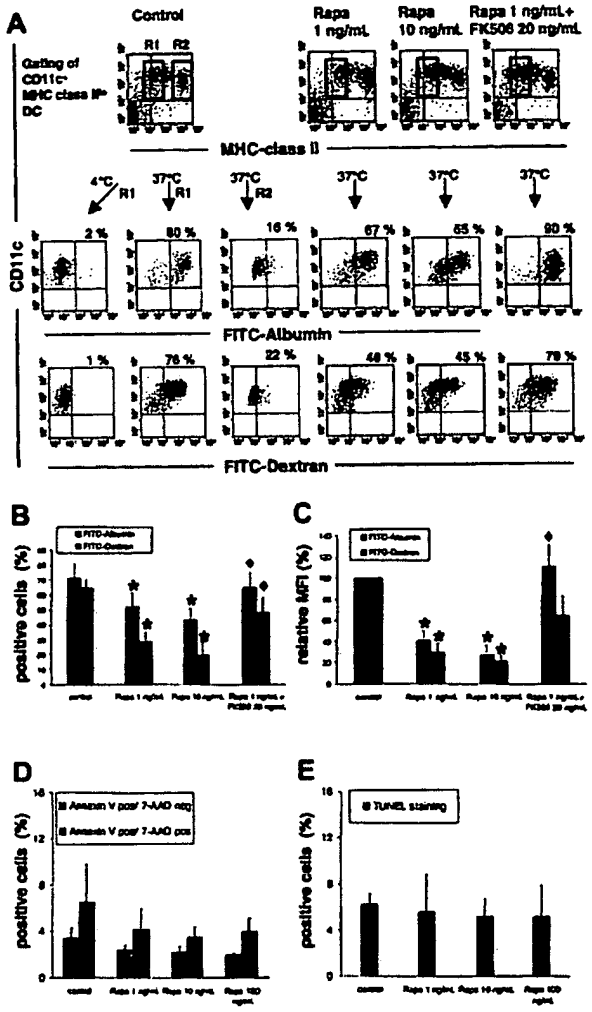


Figure 2. Rapamycin inhibits endocytosis by GM-CSF–expanded immature BM-derived DCs. (A-E) BM-derived DCs were expanded for 7 days with GM-CSF only, as described in "Study design"; rapamycin (Rapa) was added at day 2 (± FK506) at the concentrations indicated. (A-C) FITC-albumin and FITC-dextran internalization at 37°C and 4°C (negative control), MHC class II ($I-A^b$ β-chain), and CD11c expression were analyzed by 3-color flow cytometry. $CD11c^+$ MHC class II^{lo} cells were gated to determine endocytosis specifically in immature DCs. Regions R1 and R2 show endocytotic activity of immature MHC class II^{lo} and mature MHC class II^{hi} DCs, respectively. (A, B) Numbers indicate the percentage of $CD11c^+$ MHC class II^{lo} cells positive for the marker indicated. (B, C) Data represent mean values (± SE) of $CD11c^+$ MHC class II^{lo} cells after subtraction of background fluorescence (4°C). Differences between paired cultures were compared by the 2-tailed Student *t* test for paired samples (*$P \leq .01$ vs control; ♦ $P < .05$ vs Rapa 1). (D, E) Apoptosis of $CD11c^+$ DC was determined by annexin-V/7-AAD staining (D) and the TUNEL assay (E). Numbers represent mean values (± SE). Incidences of apoptotic (annexin-V positive/7-AAD negative or TUNEL positive) or secondary necrotic (annexin-V positive/7-AAD positive) cells were consistently lower than 10%. There was a trend toward *decreased* apoptosis/cell death in rapamycin-treated cultures, but the differences were not statistically significant. (A-C) Results are representative of 4 separate experiments. (D, E) Results are representative of 2 (TUNEL assay) and 3 (annexin-V/7-AAD assay) separate experiments.

with controls was < 42% and < 32% with respect to FITC-albumin and FITC-dextran, respectively (Figure 2C). Given that mean *trough* whole blood levels of rapamycin in patients are 17.3 ng/mL (5 mg rapamycin/d)[18] and that the free plasma fraction is 8%, these concentrations are clinically highly relevant. When rapamycin was added at day 6 of culture, it still inhibited DC endocytosis, but the overall effect was weaker (relative MFI 69% and 53% for FITC-albumin and FITC-dextran, respectively, at 5 ng/mL rapamycin).

Binding of rapamycin to FKBP12 and TOR inhibition can be antagonized in vitro by the structurally related immunophilin ligand FK506.[19,20] Whereas FK506 alone (20 ng/mL) did not interfere with DC endocytosis (data not shown), addition of 20 ng/mL FK506 to 1 ng/mL rapamycin (22.3-fold molar excess) antagonized the inhibitory effects of rapamycin, indicating that these were related to FKBP12-mediated TOR inhibition (Figures 1A, B; 2A-C). However, the antagonistic effect of FK506 was incomplete, especially with respect to mannose receptor–mediated endocytosis. Similar results were obtained over a wide range of drug concentrations (1-20 ng/mL rapamycin, 10-250 ng/mL FK506) and at different FK506/rapamycin ratios (10-55 molar). This suggested that other FKBPs might also be involved in endocytosis inhibition. One candidate is FKBP25, which has > 100 times greater binding affinity for rapamycin than FK506.[21]

Having established that rapamycin inhibited DC endocytosis, we analyzed whether this effect was due to increased apoptotic cell death. In contrast to a recent report,[22] the incidence of apoptosis at day 7 of culture was consistently low (< 10%) and was not affected significantly by rapamycin, even at a suprapharmacological dose of 100 ng/mL, as determined independently by annexin-V/7-AAD and TUNEL staining (Figure 2D, E). Similar results were obtained with GM-CSF+IL-4–expanded DCs and at day 4 of culture (data not shown). To investigate the in vivo relevance of DC endocytosis inhibition, we analyzed endocytotic activity in splenic DCs of animals that were injected with rapamycin (0.5 mg/kg/d for 10 days, ip) or vehicle and in which DCs were expanded with Flt3 ligand as we have described.[14] After 10 days, the animals were killed and FITC-albumin and FITC-dextran uptake by freshly isolated splenic $CD11c^+$ DCs was analyzed. The phenotype of

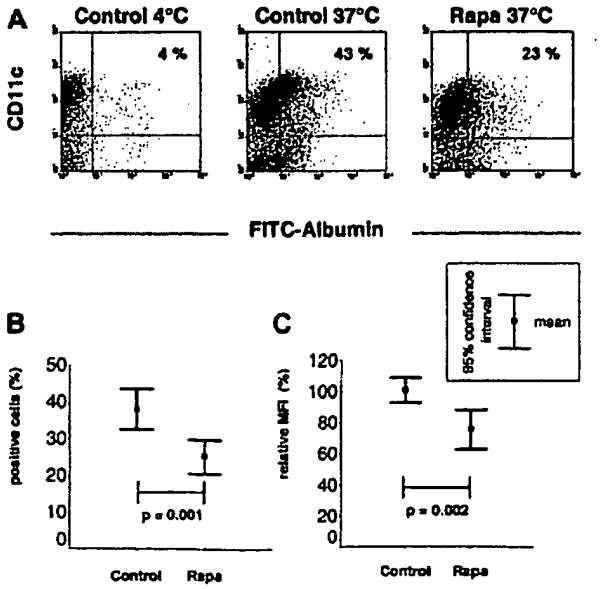


Figure 3. In vivo administration of rapamycin inhibits endocytosis by Flt3 ligand–expanded splenic DCs. (A-C) DCs were expanded in vivo with Flt-3 ligand and animals were injected with either rapamycin or vehicle, as described in "Study design." Endocytotic activity was measured by analyzing FITC-albumin uptake at 37°C and 4°C (negative control) and relative MFI in comparison with animals injected with vehicle only. Results are representative of 8 animals per group and were obtained in 5 independent experiments. Differences between the groups were compared by the 2-tailed Student *t* test for independent samples. Similar findings were obtained with respect to FITC-dextran uptake, as described in "Results and discussion."

these DCs was immature in both treatment groups. As shown in Figure 3, CD11c$^+$ DC of rapamycin-treated animals displayed significantly reduced macropinocytotic activity, with respect to both the number of positive cells and the relative MFI ($P = .001$ and $P = .002$, respectively). Similar findings were obtained with respect to FITC-dextran uptake (41.5% positive cells vs 24.8% in rapamycin-injected animals, $P < .05$; relative MFI 74.7%, $P < .05$).

The results of this study suggest that rapamycin can interfere with immune responses at a very early stage by inhibiting DC endocytosis. Thus, rapamycin targets a unique function of DCs that influences the induction of immunity against microbial pathogens (eg, *Salmonella*[23]) and allergens.[24] This effect may also suppress indirect alloantigen processing following transplantation.

The precise mechanisms by which rapamycin inhibits DC endocytosis remain to be determined. In this context, it is important to note that the Rho GTPases CDC42 and Rac that interfere with the endocytotic activity of DCs[7,8] complex with and activate the p70 S6 kinase[25] that belongs to the central signaling pathway disrupted by rapamycin.[9] In addition, rapamycin's inhibition of TOR signaling down-regulates protein translation and has been demonstrated to suppress actin synthesis.[26] Because, to our knowledge, no clinical immunosuppressant has been reported to inhibit DC endocytosis, these findings may provide further incentive for trials of rapamycin in clinical settings other than transplantation, for example, in autoimmune disease.

Acknowledgments

We thank Jan Urso for excellent technical assistance, Wyeth-Ayerst for providing rapamycin and advice, Immunex for providing Flt3 ligand, and Dr Venkataramanan for helpful discussion.

References

1. Banchereau J, Briere F, Caux C, et al. Immunobiology of dendritic cells. Annu Rev Immunol. 2000; 18:767-811.
2. Hackstein H, Morelli AE, Thomson AW. Designer dendritic cells for tolerance induction: guided not misguided missiles. Trends Immunol. 2001;22: 437-442.
3. Sallusto F, Cella M, Danieli C, Lanzavecchia A. Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: downregulation by cytokines and bacterial products. J Exp Med. 1995; 182:389-400.
4. Piemonti L, Monti P, Allavena P, et al. Glucocorticoids affect human dendritic cell differentiation and maturation. J Immunol. 1999;162:6473-6481.
5. Hackstein H, Morelli AE, Larregina AT, et al. Aspirin inhibits in vitro maturation and in vivo immunostimulatory function of murine myeloid dendritic cells. J Immunol. 2001;166:7053-7062.
6. Garrett WS, Chen LM, Kroschewski R, et al. Developmental control of endocytosis in dendritic cells by Cdc42. Cell. 2000;102:325-334.
7. West MA, Prescott AR, Eskelinen EL, Ridley AJ, Watts C. Rac is required for constitutive macropinocytosis by dendritic cells but does not control its downregulation. Curr Biol. 2000;10:839-848.
8. de Baey A, Lanzavecchia A. The role of aquaporins in dendritic cell macropinocytosis. J Exp Med. 2000;191:743-748.
9. Sehgal SN. Rapamune (RAPA, rapamycin, sirolimus): mechanism of action immunosuppressive effect results from blockade of signal transduction and inhibition of cell cycle progression. Clin Biochem. 1998;31:335-340.
10. Saunders RN, Metcalfe MS, Nicholson ML. Rapamycin in transplantation: a review of the evidence. Kidney Int. 2001;59:3-16.
11. Hong JC, Kahan BD. Sirolimus rescue therapy for refractory rejection in renal transplantation. Transplantation. 2001;71:1579-1584.
12. Shapiro AM, Lakey JR, Ryan EA, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. N Engl J Med. 2000;343:230-238.
13. Raught B, Gingras AC, Sonenberg N. The target of rapamycin (TOR) proteins. Proc Natl Acad Sci U S A. 2001;98:7037-7044.
14. Morelli AE, Antonysamy MA, Takayama T, et al. Microchimerism, donor dendritic cells, and alloimmune reactivity in recipients of Flt3 ligand-mobilized hemopoietic cells: modulation by tacrolimus. J Immunol. 2000;165:226-237.
15. Grabbe S, Kampgen E, Schuler G. Dendritic cells: multi-lineal and multi-functional. Immunol Today. 2000;21:431-433.
16. Metlay JP, Witmer-Pack MD, Agger R, Crowley MT, Lawless D, Steinman RM. The distinct leukocyte integrins of mouse spleen dendritic cells as identified with new hamster monoclonal antibodies. J Exp Med. 1990;171:1753-1771.
17. Inaba K, Inaba M, Romani N, et al. Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. J Exp Med. 1992;176:1693-1702.
18. MacDonald A, Scarola J, Burke JT, Zimmerman JJ. Clinical pharmacokinetics and therapeutic drug monitoring of sirolimus. Clin Ther. 2000; 22(suppl B):B101-B121.
19. Kuo CJ, Chung J, Fiorentino DF, Flanagan WM, Blenis J, Crabtree GR. Rapamycin selectively inhibits interleukin-2 activation of p70 S6 kinase. Nature. 1992;358:70-73.
20. Dumont FJ, Melino MR, Staruch MJ, Koprak SL, Fischer PA, Sigal NH. The immunosuppressive macrolides FK-506 and rapamycin act as reciprocal antagonists in murine T cells. J Immunol. 1990;144:1418-1424.
21. Jin YJ, Burakoff SJ, Bierer BE. Molecular cloning of a 25-kDa high affinity rapamycin binding protein, FKBP25. J Biol Chem. 1992;267:10942-10945.
22. Woltman AM, de Fijter JW, Kamerling SW, et al. Rapamycin induces apoptosis in monocyte- and CD34-derived dendritic cells but not in monocytes and macrophages. Blood. 2001;98:174-180.
23. Niedergang F, Sirard JC, Blanc CT, Kraehenbuhl JP. Entry and survival of *Salmonella typhimurium* in dendritic cells and presentation of recombinant antigens do not require macrophage-specific virulence factors. Proc Natl Acad Sci U S A. 2000;97: 14650-14655.
24. Noirey N, Rougier N, Andre C, Schmitt D, Vincent C. Langerhans-like dendritic cells generated from cord blood progenitors internalize pollen allergens by macropinocytosis, and part of the molecules are processed and can activate autologous naive T lymphocytes. J Allergy Clin Immunol. 2000;105:1194-1201.
25. Chou M, Blenis J. The 70 kDa S6 kinase complexes with and is activated by the Rho Family G proteins Cdc42 and Rac1. Cell. 1996;85:573-583
26. Miyamoto S, Safer B. Immunosuppressants FK506 and rapamycin have different effects on the biosynthesis of cytoplasmic actin during the early period of T cell activation. Biochem J. 1999; 344:803-812.

International Immunology, Vol. 10, No. 6, pp. 749–755

IL-12 increases CD80 expression and the stimulatory capacity of bone marrow-derived dendritic cells

Peter Kelleher and **Stella C. Knight**

Antigen Presentation Research Group, Imperial College School of Medicine, Northwick Park Institute for Medical Research, Watford Road, Harrow, Middlesex HA1 3UJ, UK

Keywords: adjuvant, autocrine effects, IL-12, mixed leukocyte reaction

Abstract

Dendritic cells (DC) are potent antigen-presenting cells derived from CD34 bone marrow stem cells. They undergo a series of maturational steps that allow them to stimulate primary T cell responses. Several cytokines are known to contribute to this process. In this study murine DC maturing from bone marrow progenitors under the influence of granulocyte macrophage colony stimulating factor and tumour necrosis factor-α were found to produce IL-12 as measured by ELISA and by flow cytometry to detect intracellular cytokine. Administration of additional IL-12 from day 3 to 7 of culture altered the function and phenotype of DC; enhanced stimulation of T cell proliferation by DC in allogeneic mixed leukocyte reactions was associated with an increase in the surface expression of CD80 on DC. These effects were dose dependent, and were consistently seen with IL-12 at 25 ng/ml and were less marked with IL-12 at 50 ng/ml. These results show that IL-12 is both produced by DC and can increase their stimulatory capacity. The findings suggest that there may be an autocrine effect of IL-12 on DC maturation and function.

Introduction

Bone marrow-derived dendritic cells (DC) are specialized to acquire and process antigens in peripheral tissues. They transport antigen via the afferent lymphatics to lymph nodes, and mature and up-regulate histocompatibility antigens and co-stimulatory molecules such as CD40, CD80 and CD86. In the lymph nodes, DC have less capacity to process antigen but possess the unique ability to activate naive T cells. DC are also efficient stimulators of secondary T cell immune responses (1).

IL-12 is a heterodimeric cytokine composed of covalently linked p35 and p40 subunits encoded on two separate genes (2). The p35 mRNA is constitutively expressed by a variety of cell types, whereas p40 expression is correlated with the production of bioactive p70 by antigen-presenting cells (3). IL-12 is secreted in three main forms, i.e. the p70 heterodimer, p40 homodimers and p40 monomers. The function of the p40 moieties is not yet established *in vivo*. However, p70 enhances the proliferation and cytolytic activity of NK cells and T cells, and stimulates IFN-γ production by these cells. DC produce IL-12 which promotes the development of CD4 T_h1 cells from naive T cells (4–6). CD40 ligation on DC enhances both their maturation and stimulatory capacity in the mixed lymphocyte reaction (MLR) and induces the production of IL-12 in these cells (7–9). Since cytokines can amplify the antigen-presenting function of DC, it is possible that IL-12 could play a direct role in this process.

The aims of this study were (i) to see if bone marrow-derived DC produce IL-12 at different time points during maturation, and (ii) whether IL-12 administration during DC maturation altered cell numbers, phenotype and function.

Materials

Mice

Specific pathogen-free female BALB/c and CBA mice, 6–10 weeks old, were obtained from Northwick Park Institute for Medical Research.

Media and reagents for cell culture

The culture medium (CM) was RPMI 1640 (Dutch modification; Sigma, Poole, UK) supplemented with 10% FCS (Gibco, Paisley, UK), 100 U Penicillin (Gibco), 100 μg/ml streptomycin

Correspondence to: S. C. Knight

Transmitting editor: M. Feldmann

Received 13 June 1997, *accepted* 12 February 1998

(Gibco), 2 mM glutamine (ICN Flow, Irvine, UK), 2 mercaptoethanol 10^{-5} M (Sigma). Wash medium was identical to the CM but contained bicarbonate-buffered RPMI 1640 instead of the Dutch modification. Granulocyte macrophage colony stimulating factor (GM-CSF) and tumour necrosis factor (TNF)-α (R & D Systems, Oxford, UK) were used at concentrations of 100 and 50 U/ml respectively. IL-12 was a generous gift from Dr M. Gately (Roche Discovery, Nutley, NJ) and was used at 10–50 ng/ml.

DC

DC were derived from bone marrow precursors as previously described (10). Briefly, bone marrow was obtained from the femura and tibiae of BALB/c mice. Mononuclear cells were isolated by centrifuging on Lympholyte M (Cedarlane, Hornby, Ontario, Canada). The interface cells were collected, washed and suspended in CM at a concentration of 10^6 cells/ml, and GM-CSF and TNF-α were added. After a 2–4 day incubation the non-adherent cells were centrifuged at 600 *g* over metrizamide (Nyegaard, Oslo, Norway; 13.7% w/v) to collect the low-density cell population (LDC) in which DC are found. The LDC were resuspended in CM at a cell concentration of 5–10×10^5 cells/ml supplemented with GM-CSF and TNF-α. IL-12 at a concentration from 10–50 ng/ml was then added to the cultures. The medium was changed every 2–3 days and IL-12 together with the other two cytokines were replenished over the next 5 days. After a total of 10–14 days of culture the non-adherent cell population was again centrifuged over a metrizamide gradient, and the LDC washed and suspended in CM. DC numbers were estimated by light microscopy and Trypan blue was used to estimate cell viability. Normal cell viability was 95–100%.

Flow cytometry

The DC were labelled with directly conjugated FITC mAb: mouse I-A^d (clone AMS 32.1, IgG2b), mouse H-$2D^d$ (clone 34-2-12, IgG2a), hamster anti-mouse CD11c (clone HI3, hamster IgG), hamster anti-mouse CD54 (clone 3E2, hamster IgG) and rat anti-mouse CD11a (clone C71/16, rat IgG2a). Biotinylated mAb employed were: rat anti-mouse CD40 (clone 3/23, rat IgG2a), rat anti-mouse CD80 (clone 1G10, rat IgG2a), rat anti-mouse CD86 (GL1, IgG2a), rat anti-mouse CD8 (clone ABC, rat IgG2a), hamster anti-mouse CD95 (clone Jo2, hamster IgG) and rat anti-mouse IL-12 (clone C17.15, rat IgG2a). The FITC-conjugated antibodies were purchased from PharMingen (San Diego, CA). The biotinylated IL-12 antibody was obtained from Genzyme (Cambridge, MA) and the other biotin antibodies were obtained from PharMingen. Avidin–FITC and streptavidin–phycoerythirn (PE) (Becton Dickinson, Mountain View, CA) were used to label the biotinylated antibodies.

Twenty percent FCS in FACScan buffer was used to prevent non-specific antibody binding. FITC-conjugated and biotinylated mAb were added to the LDC and the samples were left on ice for 30 min. The cells were then washed in FACScan buffer and avidin–FITC was added. After 20 min these samples were washed and 50 µl of 1% paraformaldehyde added for overnight fixation. Fluorescence profiles were generated on a FACScan flow cytometer (Becton Dickinson). Histogram and density plots were produced by the CellQuest (version 1.0) software package. Viable DC were selected after gating on forward and side scatter with dead cells excluded by propidium iodide staining. The cells which were selected in the gate set around the DC cluster expressed high levels of MHC class II, and were stained by murine markers for DC such as NLDC-145, 33D1 and CD11c as previously described (10). Several antigens were not detected including CD19 (B cells), CD3ε (T cells) and CD115 (macrophages). Ninety percent of the selected cells expressed the murine-specific DC marker NLDC-145. The user parameters set for experiments involving control and IL-12-treated DC were as follows: FSC linear 5.67, SSC log 242 and FL1 log 460.

Intracellular flow cytometry

Monensin at a concentration of 1.5 mM was added to 5–10×10^5 LDC to ensure intracellular cytokine retention. The LDC/monensin preparation was incubated for 6 h at 37°C. The cells were then washed twice in FACScan buffer containing 20% FCS. Cytoperm A (Serotec, Oxford, UK) was added, the cells kept at room temperature for 15 minutes for fixing and then washed again in FACScan buffer with 20% FCS. Cells were then exposed to permeabilizing agent, Cytoperm B (Serotec), at room temperature for 15 min. The cells were then double labelled at room temperature for the p40 subunit of IL-12 (rat anti-mouse IgG2a; Genzyme) and murine DC-specific markers NLDC-145 (cell line rat IgG2a) and 33D1 (cell line rat IgG2b). The fluorescent cytokine profiles were generated on the FACScan flow cytometer as described above. The user parameters for these experiments were FSC linear 5.67, SSC log 242, FL1 log 440, FL2 log 475, and compensation FL1–FL2 0.6 and FL2–FL1 29.5.

Lymphocyte proliferation assays

Lymphocyte proliferation assays were performed in 20 µl hanging drop cultures on inverted Terasaki plates (11). CBA lymph nodes were pressed through a wire gauge in medium, washed once and used as responder cells for the BALB/c-derived bone marrow DC. On day 3 the cultures were pulsed with 1 µg/ml [^{3}H]thymidine with a sp. act. of 2 Ci/mM (Amersham International, Amersham, UK) for 2 h and then transferred by blotting on to filter discs. The thymidine uptake into proliferating cells was measured in the acid-insoluble fraction on the filter discs in a liquid scintillation counter (Canberra Packard 2000 CA Tri-Carb). This technique gives values that are lower than usual in the 200 µl culture systems, but they reflect accurately the DNA synthesis occurring in these cultures. Data points for each experiment shown represent the mean c.p.m. of triplicate cultures. ANOVA and Student's *t*-test were used to identify significant effects.

Cytokine measurement

IL-12 p70, IL-12 p40, IFN-γ and IL-4 levels were determined by the Intertest solid-phase ELISA kit (Genzyme). Microtiter plates were coated with specific antibodies to capture the cytokine of interest in cell culture supernatants. A second layer antibody was then added. The detection of bound cytokine was achieved using a horseradish peroxidase-conjugated antibody and a colour substrate. Cytokine concentrations were determined with a standard curve derived from known amounts of the relevant cytokine using absorbance

readings at 450 nm on a spectrophotometer. There was no interference from p40 in the culture supernatants with the p70 heterodimer. The lower limits of detection for IL-12 were 10 pg/ml for p40 and 5 pg/ml for p70 and the other cytokines.

Results

DC derived from bone marrow preparations secrete IL-12

The presence of IL-12 in supernatants from maturing LDC (10^6 cells) was assessed on day 5 and 8 of culture. IL-12 p40 was found in excess of IL-12 p70 as previously described (6). The values on day 5 were 120 pg/ml of IL-12 p40 and 20 pg/ml of IL-12 p70. Three days later the amounts of IL-12 p40 and IL-12 p70 had increased to 150 and 24 pg/ml respectively. Similar values for IL-12 were found on repeat experiments. There were no lymphocytes observed in these cultures on light microscopy, which was confirmed on flow cytometry using the T and B cell markers CD3ε and CD19. IFN-γ and IL-4 and IL-10 were not detected in culture supernatants at these time points.

Flow cytometry was used to detect intracellular cytokine production by DC derived from bone marrow precursors. IL-12 p40 was identified in cells which express DC markers (Fig. 1). A higher proportion of the cells labelled with NLDC than 33D1 as previously described (10). Almost all the cells producing IL-12 were positive for NLDC and most labelled with 33D1. Eighteen percent of the NLDC-labelled cells and 38% of the 33D1 positive cells were positive for IL-12 p40. Non-specific intracellular binding by the p40 antibody was excluded by showing that IL-12 p70 in excess inhibited intracellular p40 labelling and there was no labelling for IL-12 p40 in the absence of monensin (Fig. 1). In addition, an isotype antibody (rat anti-mouse IgG2a) was also used as a negative control for IL-12 p40 (data not shown).

IL-12-treated DC exhibit differences in cell surface phenotype and function

IL-12 administration did not alter the proportion or numbers of DC derived from bone marrow progenitors as assessed by light microscopy and flow cytometry (data not shown); there was no evidence for any toxic effects of IL-12 at the highest dose. The effect of IL-12 administration at 10, 25 and 50 ng/ml on the surface phenotype of DC after 10–14 days of culture was examined by flow cytometry. A most striking finding was that 25 ng/ml of IL-12 up-regulated cell surface expression of CD80 on DC. The geometric mean fluorescence intensity of the control DC was significantly higher following IL-12 treatment with 25 ng/ml, e.g. 39.1 compared with 59.9 (Fig. 2). The influence of 50 ng/ml of IL-12 on CD80 expression on DC was more variable; in some experiments (three out of 10) CD80 expression was also increased (data not shown). There were no reproducible differences in CD80 expression with the lowest dose of IL-12 (10 ng/ml) and mean fluorescence from labelling with a number of other antibodies showed no reproducible differences with any dose of IL-12 (Fig. 2).

DC treated with IL-12 at 25 ng/ml stimulated higher levels of T cell proliferation than untreated cells (Fig. 3). DC treated with 50 ng/ml usually did not increase T cell proliferation in the MLR (seven out of 10 experiments), although in the three

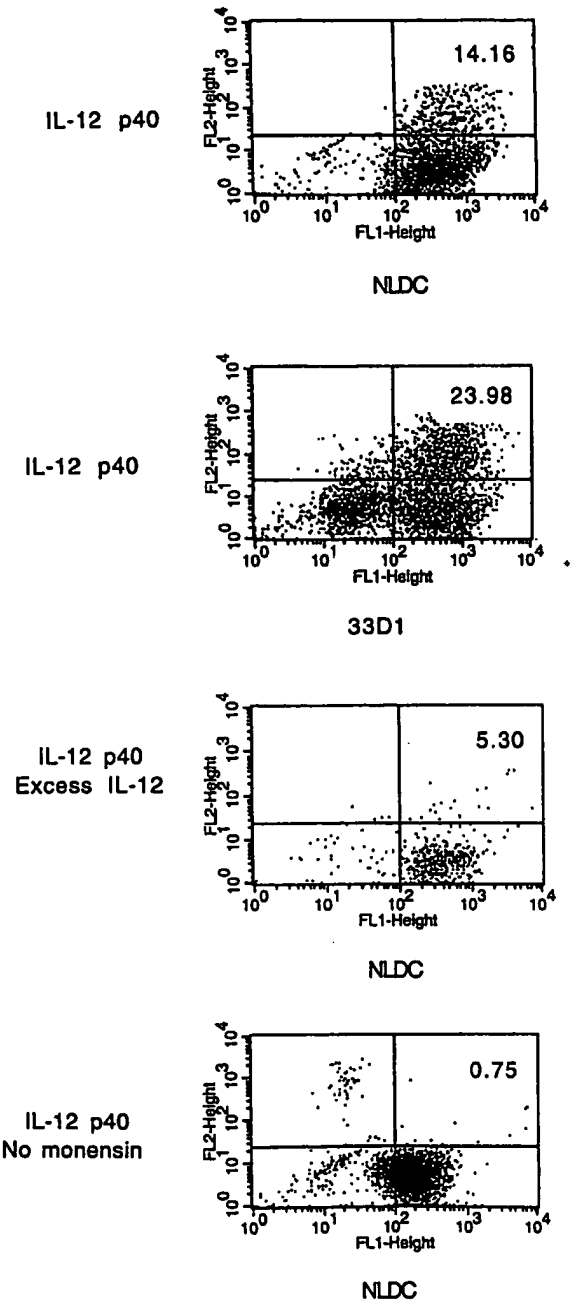


Fig. 1. Flow cytometry to measure intracellular IL-12 in DC was performed using double labelling with a biotinylated IL-12 p40 antibody and NLDC or 33D1 as markers for DC as indicated on the axes. Controls for IL-12 p40 labelling were DC incubated with excess IL-12 p70 and cells prepared in the absence of monensin which both showed little labelling, and the results are presented for the cells labelled with NLDC. The percentage of the total cell population that was positive for both markers is indicated.

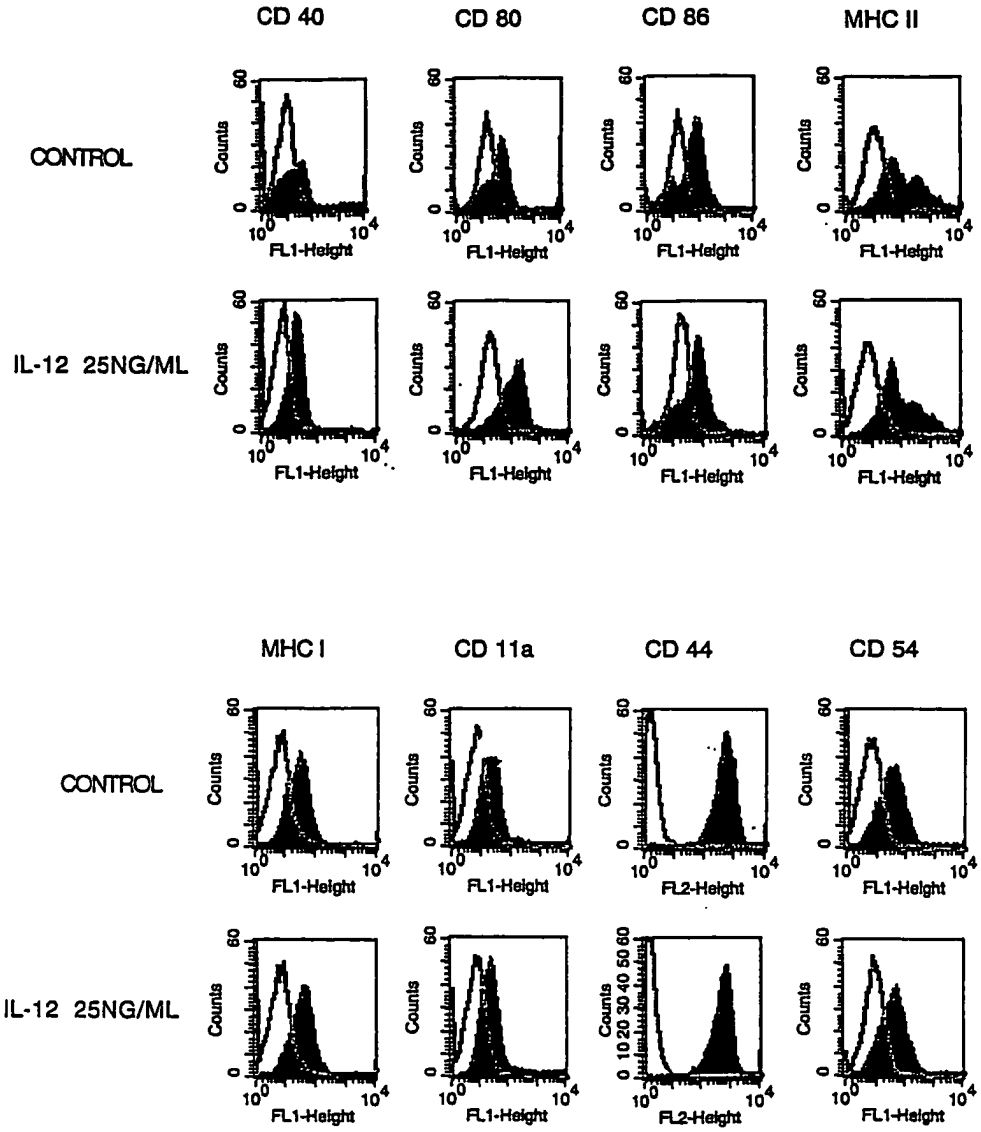


Fig. 2. DC derived from bone marrow progenitors were treated from 3–7 days of culture with medium control or IL-12 at 25 ng/ml. After a total of 10 days of culture DC were labelled with different antibodies. The number of cells (ordinate) with different levels of fluorescent labelling (abscissa) are shown for cells exposed to an isotype control (open histogram) or to a specific antibody (filled histogram). Representative of three experiments performed.

experiments where CD80 expression was elevated there was an increase in the stimulatory capacity of these cells. Results from one experiment where IL-12 treatment at 25 and 50 ng/ml both increased expression of CD80 T cell proliferation are shown in Fig. 3(B). The effect of IL-12 dosimetry is indicated as even in this experiment IL-12 treatment at 50 ng/ml was less effective than IL-12 at 25 ng/ml in T cell stimulation with 1000 DC. IL-12 treatment at 10 ng/ml was associated with a reduction in T cell proliferation in the MLR proliferation but this did not reach statistical significance (Fig. 3B).

Discussion

IL-12 administration at 25 ng/ml to maturing LDC increased the surface expression of CD80 which was associated with an increase in stimulation of T cell proliferation by DC in the MLR. This work also confirms the finding that IL-12 can be secreted by DC in cultures of LDC developing from bone marrow cells (6). The results raise the concept that IL-12 produced by the DC themselves could influence their maturation; slight variability in the level of endogenously

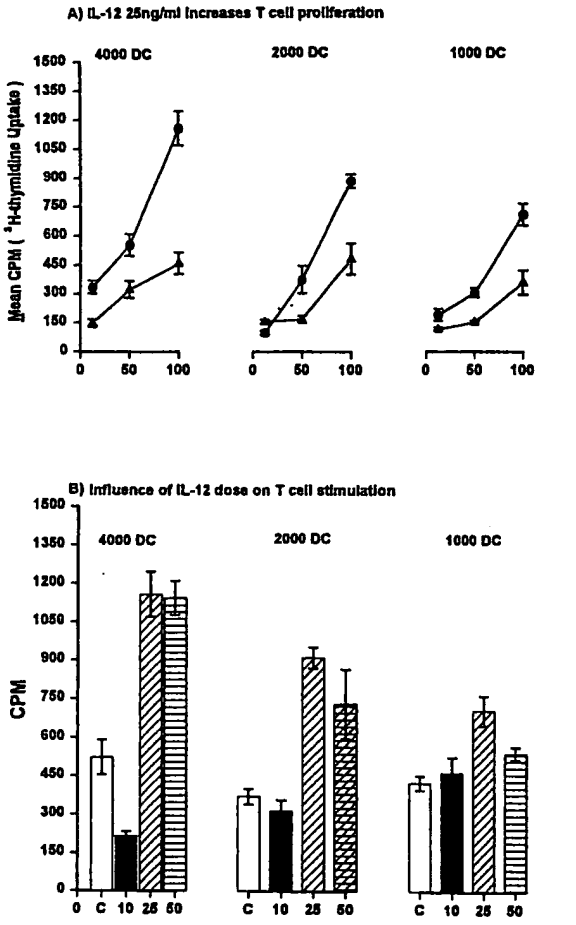


Fig. 3. (A) DC derived from bone marrow progenitors were treated with medium or IL-12 at 25 ng/ml from day 3 to 7 of culture. After a total of 10 days culture DC were incubated with CBA lymph nodes responder cells in 20 µl 'hanging drop' cultures. Lymphocyte proliferation was determined from the uptake of [^{3}H]thymidine for 2 h on day 3 of culture. The graph shows results from one of four similar experiments: ▲, control DC; ●, IL-12-treated DC. Lymph node cells alone gave counts of <50 c.p.m. Differences in stimulation of T cell proliferation by control DC and IL-12-treated DC were statistically significant in all experiments ($P < 0.05$). (B) DC were treated with different doses of IL-12 and the MLR were performed as described above. Graph shows the response of 10^5 CBA lymph node responder cells to stimulation by different doses of control DC (C) or DC treated with IL-12 at 10 (10), 25 (25) and 50 (50) ng/ml. Similar responses were observed with lower lymph node cell concentrations. The mean fluorescence for CD80 labelling of DC used in this lymphocyte proliferation assay was control, 52.8; IL-12, 10 ng/ml, 32.5; IL-12, 25 ng/ml, 96.5; and IL-12, 50 ng/ml, 72.9.

produced IL-12 might contribute to the greater variability in the effects of higher doses of added IL-12.

There is conflicting data on whether maturing bone marrow DC secrete IL-12 independently of T cell contact. Heufler and colleagues detected IL-12 p40 and p70 in supernatants of bone marrow-derived DC, whereas Winzler and her associates only detected bioactive IL-12 after antigen presentation to T cells (6,12). One reason for this discrepancy may well lie in the methods used to generate DC from bone marrow progenitors as DC in this and the Heufler study were more mature than the cells used by Winzler; the ability of DC to produce IL-12 may be proportional to the maturational stage of these cells. This current study shows that IL-12 administration *in vitro* can influence the maturation and immunostimulatory function of DC independently of T cell contact.

IL-12 is likely to act directly on DC since there were few contaminating cells. In addition, DC are the principal stimulatory cells in the MLR and macrophages show little or no activity in this lymphocyte proliferation assay supporting the evidence of an effect directly on DC (13,14). Some of the differences in T cell proliferation observed with the highest doses of IL-12 may again reflect underlying variation in endogenous IL-12 production by DC in different cultures. In addition to any autocrine effects of IL-12 on DC function, the amount of IL-12 that these cells produce may influence the magnitude of T cell proliferation observed in MLR. The mechanisms by which exogenous and endogenous IL-12 may interact to enhance the antigen-presenting capacity of DC remains to be determined.

This work adds IL-12 to the growing numbers of cytokines that can influence antigen presentation by DC. Short-term culture with GM-CSF enhances the stimulatory capacity of skin DC in the MLR (15,16). TNF-α enhances the maturation and allostimulatory capacity of DC in long-term murine bone marrow cultures (12). IL-1α administration has been shown to enhance T cell proliferation in the MLR by spleen, thymus and skin DC (15,17,18). Since IL-12 is produced by DC, it may have an autocrine effect on DC maturation and function in the MLR; this contrasts with IL-1α, which is neither produced by DC nor detected in allogeneic MLR (19,20).

Two previous studies had suggested that IL-12 may influence DC antigen-presenting function to T cells, although neither provided any experimental evidence for this. Using BALB/c mice transgenic for $V_\alpha 11$ and $V_\beta 3$ as a model to examine the development of T_h1 cells it was proposed that IL-12 and IFN-γ could provide a stimulus to antigen-presenting cells to promote IFN-γ secretion by CD4 lymphocytes (21). Secondly, Enk and colleagues proposed that IL-12 release from human keratinocytes after exposure to allergen could modify antigen presentation by Langerhan cells during primary immune responses in the skin (22).

Changes in cell surface expression of CD80 are known to influence T cell stimulation by DC. Administration of GM-CSF *in vitro* to Langerhans cells and other tissue DC increases their ability to stimulate T cell proliferation in allogeneic MLR via CD80 up-regulation (23). On the other hand, IL-10 can inhibit antigen presentation by Langerhans cells to CD4 T_h1 lymphocytes in BALB/c mice by suppressing their CD80 expression (24). One possible explanation for the increase in T cell proliferation by IL-12-treated DC is that T_h1 cells are dependent on CD80 for their continued stimulation, as it has been shown that CD80 synergizes with IL-12 to produce IL-2 receptor in murine T_h1 clones (25). It needs to be established how IL-12 treatment increases the expression of CD80 on DC. Whether IL-12 acts directly on DC via its receptor and Jak–STAT family of transcription molecules or indirectly

through the secretion of IFN-γ and TNF-α remains to be determined.

The action of IL-12 on DC function may partially explain its mechanism of action in the treatment of infectious diseases and cancer as well as its role in the induction of organ-specific autoimmune diseases. Addition of IL-12 to peripheral blood mononuclear cells obtained from HIV-infected individuals restores the proliferative responses by T cells to a number of different stimuli (26). As DC infection by HIV can lead to reduced T cell proliferation, it has been proposed that IL-12 may be acting on DC to restore their function in this disease (27). The possibility that IL-12 can influence antigen presentation by DC was also suggested by two papers which showed that IL-12 treatment *in vitro* can enhance the ability of DC to induce anti-tumour activity *in vivo* (28,29). IL-12 has also been implicated in the development of autoimmune joint and central nervous system disease (30). Substitution of *Mycobacterium tuberculosis* by IL-12 can induce severe arthritis in DBA/1 mice injected with Freund's incomplete adjuvant and type II collagen, suggesting that IL-12 is acting on antigen-presenting cells (31). Increased expression of IL-12 p40 and CD80 has been described in neuronal plaques of patients with multiple sclerosis, which again suggests that IL-12 may also be acting on antigen-presenting cells in addition to T cells in this disease (32). The amount of endogenous IL-12 production by DC may have contributed to the effects observed with IL-12 in these studies. Localized production of IL-12 by DC may have either additive or synergistic effects on the systemic administration of IL-12. This could be an important contributor to the outcome of IL-12 therapy in infectious diseases and cancer, and susceptibility to autoimmune diseases.

IL-12 has complex effects on haematopoesis. *In vitro* administration of IL-12 directly increases the numbers of CD34 bone marrow stem cells and their myeloid progeny in synergy with other growth factors such as stem cell factor (SCF) or IL-3 (33). IL-12 *in vivo* decreases peripheral blood counts and bone marrow cellularity largely via IFN-γ induction (34). In this *in vitro* study IL-12 had no significant effects on DC numbers derived from bone marrow progenitors. The differences between this work and other *in vitro* studies may be that IL-12 shows little synergy with GM-CSF in promoting haematopoesis. Further studies examining IL-12 administration in combination with SCF are needed to determine if this cytokine can alter *in vitro* DC yields from bone marrow-derived precursors.

In conclusion, this study demonstrates that bone marrow-derived DC secrete IL-12 p40 and p70, and that IL-12 administration can alter their phenotype and function. The effect of IL-12 on antigen presentation of DC could be used to enhance the effectiveness of vaccines, and could also underlie some of its therapeutic action in the treatment of infectious diseases and cancer.

Abbreviations

DC	dendritic cell
GM-CSF	granulocyte macrophage colony stimulating factor
LDC	low-density cell population
MLR	mixed lymphocyte reaction
SCF	stem cell factor
TNF	tumor necrosis factor

References

1 Knight, S. C. and Stagg, A. J. 1993. Antigen-presenting cells types. *Curr. Opin. Immunol.* 5:374.
2. Schoenhaut, D. S., Chua, A. O., Wolitzky, A. G., Quinn, P. M., Dwyer, C. M., McComas, W, Familletti, P. C., Gately, M. K. and Gubler, U. 1992. Cloning and expression of murine IL-12. *J. Immunol.* 148:3433.
3 Trinchieri, G. 1995. Interleukin-12: a proinflammatory cytokine with immnunoregulatory functions that bridge innate resistance and antigen specific adaptive immunity. *Annu. Rev. Immunol.* 13:251.
4 Kanangat, S., Nair, S., Sam Babu, J. and Rouse B. T. 1995. Expression of cytokine in murine splenic dendritic cells and better induction of T cell derived cytokines by dendritic cells than by macrophages during *in vitro* costimulation assay using specific antigens. *J. Leuk. Biol.* 57:310.
5 Macatonia, S. E., Hosken, N. A., Litton, M., Vieira, P., Hsieh, C.-S., Culpepper, J. A., Wysocka, M., Trinchieri, G., Murphy, K. M. and O'Garra, A. 1995. Dendritic cells produce IL-12 and direct the development of TH1 cells from naive CD 4 T cells. *J. Immunol.* 154:5071.
6 Heufler, C., Koch, F., Stanzl, U., Topar, G., Wysocha, M., Trinchieri, G., Enk, A., Steinman, R. M., Romani, N. and Schuler, G. 1996. Interleukin-12 is produced by dendritic cells and mediates T helper 1 development as well as interferon-γ production by T helper 1 cells. *Eur. J. Immunol.* 26:659.
7 Caux, C., Massacrier, C., Vanbervliet, B., Duboois, B., Van Kooten, C., Durand, I. and Banchereau, J. 1994. Activation of human dendritic cells through CD40 cross linking. *J. Exp. Med.* 180:1263.
8 Koch, F., Stanzl, U., Jennewein, P., Janke, K., Heufler, C., Kampgen, E, Romani, N. and Schuler, G. 1996. High level IL-12 production by murine dendritic cells: upregulation via MHC II and CD 40 molecules and downregulation by IL-4 and IL-10. *J. Exp. Med.* 184:741.
9 Cella, M., Scheidegger, X., Palmer-Lehmann, X., Lane, P., Lanzavecchia, A. and Alber, G. 1996. Ligation of CD40 on dendritic cells triggers the production of high levels of interleukin-12 and enhances T cell stimulatory capacity: T-T help via APC activation. *J. Exp. Med.* 184:747.
10 Inaba, K., Inaba, M., Romani, N., Aya, H., Deguchi, M., Ikehara, S., Muramatsu, S. and Steinman, R. M. 1992. Generation of large numbers of numbers of dendritic cells from mouse bone marrow cultures with supplemented with granulocyte/macrophage colony-stimulating factor. *J. Exp. Med.* 176:1693.
11 Knight, S. C. 1988. Lymphocyte proliferation assays. In Klaus, G. G. B., ed., *Lymphocytes: A Practical Approach*, p. 189. IRL Press, Oxford.
12 Winzler, C., Rovere. P., Rescigno, M., Granucci, F., Penna, G., Adorini, L., Zimmermann, V. S, Davoust, J. and Ricciardi-Castagnoli, P. 1997. Maturation stages of mouse dendritic cells in growth factor-dependent long-term cultures. *J. Exp. Med.* 185:317.
13 Steinmann, R. M., Gutchinov, B., Witmer, M. D. and Nussenzweig, M. C. 1983. Dendritic cells are the principal stimulators of the primary mixed leucocyte reaction in mice. *J. Exp. Med.* 157:613.
14 Klinkert, W. E. F., LaBadie, J. H. and Bowers, W. E. 1982. Accessory and stimulating properties of dendritic cells and macrophages isolated from various rat tissues. *J. Exp. Med.* 156:1.
15 Heufler, C., Koch, F. and Schuler, G. 1987. Granulocyte/Macrophage colony strimulating factor and interleukin 1 mediate the maturation of murine epidermal Langerhans cells into potent immunostimulatory dendritic cells. *J. Exp. Med.* 167:700.
16 Witmer-Pack, M. D., Olivier, W., Valinsky, J., Schuler, G and Steinman, R. M. 1987. Granulocyte/Macrophage colony stimulating factor is essential for the viability and function of cultured murine epidermal Langerhans cells. *J. Exp. Med.* 166:1484.

17 Koide, S. L. and Steinmann R. M. 1987. Interleukin 1 enhances T-dependent immune responses by amplifying the function of dendritic cells. *J. Exp. Med.* 165:515
18 Inaba, K., Witmer-Pack, M. D., Inaba, M., Muramatsu, S. and Steinmann, R. M. 1988. The function of Ia$^+$ dendritic cells and Ia$^-$ dendritic cell precursors in thymocyte mitogenesis to lectin and lectin plus interleukin 1. *J. Exp. Med.* 1988 167:149.
19 Koide, S. and Steinman, R. M. 1987. Induction of murine interleukin 1: stimuli and responsive cells. *Proc. Natl Acad. Sci. USA* 84:3802.
20 McKenzie, J. L., Prickett, T. C. R. and Hart, D. N. J. 1989. Human dendritic cells stimulate allogeneic T cells in the absence of IL-1. *Immunology* 67:290.
21 Seder, R. A,. Gazzinelli, R., Sher, A. and Paul, W. E. 1993. Interleukin-12 acts directly on CD4$^+$ T cells to enhance priming for interferon-γ production and diminishes interleukin 4 inhibition of such priming. *Proc. Natl Acad. Sci. USA* 90:10188.
22 Muller, G., Saloga, J., Germann, T., Bellinghausen, I., Mohamadzadeh, M., Knop, J. and Enk, A. H. 1994. Identification and induction of human keratinocyte-derived IL-12. *J. Clin. Invest.* 94:1799.
23 Larsen, V. P., Ritchie, S. C., Hendrix, R., Linsley, P. S., Hathcock, K. S., Hodes, R. J., Lowry, R. P. and Pearson, T. C. 1994. Regulation of immunostimulatory function and costimulatory molecule (B7-1 and B7-2) expression on murine dendritic cells. *J. Immunol.* 152:5208.
24 Ozawa, H., Aiba, S., Nakagawa, S. and Tagami, H. 1996. Interferon-γ and interleukin-10 inhibit antigen presentation by Langerhans cells for T helper type 1 cells by suppressing their CD80 (B7-1) expression. *Eur. J. Immunol.* 26:648.
25 Igarashi, O., Yanagida, T., Azuma, M., Okumura, K. and Nariuchi, H. 1996. B7-1 synergises with interleukin-12 in interleukin-2 receptor 2 α expression by mouse T helper 1 clones. *Eur. J. Immunol.* 26:300.
26 Clerici, M., Lucey, D. R., Berzofsky, J. A., Pinto, L. A., Wynn, T. A., Blatt, S. P., Dolan, M. J., Hendrix, C. W., Wolf, S. F. and Shearer, G. M. 1993. Restoration of HIV-specific cell-mediated immune responses by interleukin-12 *in vitro*. *Science* 262:1721.
27 Knight, S. C. 1996. Bone marrow derived dendritic cells and the pathogenesis of AIDS. *AIDS* 10:807.
28 Bianchi, R., Grohmann, U., Belladonna, M. L., Silla, S., Fallarino, F., Ayroldi, E., Fioretti, M. C. and Pucetti, P. 1996. IL-12 is both required and sufficient for initiating T cell reactivity to a Class I-restricted tumour peptide (P815AB) following transfer of P815AB-pulsed dendritic cells. *J. Immunol.* 157:1589.
29 Grohmann, U., Bianchi, R., Ayroldi E., Belladonna, M. L., Surace, D., Fioretti, M. C. and Pucetti, P. 1997. A tumor-associated and self antigen peptide presented by dendritic cells may induce T cell anergy *in vivo*, but IL-12 can prevent or revert the anergic state. *J. Immunol.* 158:3593.
30 Trembleau, S., Germann, T., Gately, M. K. and Adorini, L. 1995. The role of IL-12 in the induction of organ specific autoimmune diseases. *Immunol. Today* 16:383.
31 Germann, T., Szeliga, J., Hess, H., Storkel, S., Podlaski, F. J., Gately, M. K., Schmitt, E. and Rude, E. 1995. Administration of Interleukin 12 in combination with type II collagen induces severe arthritis in DBA/1 mice. *Proc. Natl Acad. Sci. USA* 92:4823.
32 Windhagen, A., Newcombe, J., Dangold, F., Strand, C., Woodroofe, M. N., Cuzner, M. L. and Hafler, D. A. 1995. Expression of costimulatory molecules B7-1 (CD80), B7-2 (CD86) and interleukin 12 cytokine in multiple sclerosis lesions. *J. Exp. Med.* 182:1985.
33 Jacobsen, S. E. W., Veiby, O. P. and Smeland, E. B. 1993. Cytotoxic lymphocyte maturation factor (Interleukin 12) is a synergistic growth factor for hematopoietic stem cells. *J. Exp. Med.* 178:413.
34 Eng, V. M., Carr, B. D., Schnyder, B., Lorenz, M., Lugli, S., Aguet, M., Anderson, T. D., Ryffel, B. and Quesniaux, V. F. 1995. The stimulatory effects of interleukin (IL)-12 on hematopoiesis are antagonised by IL-12 induced interferon-γ *in vivo*. *J. Exp. Med.* 181:1893.